# NATURE

*A Weekly Journal of Science*

Vol. 184, No. 4684 SATURDAY, AUGUST 8, 1959 2s. 6d.



OHIO STATE UNIVERSITY LIBRARY
OCT 28 1959



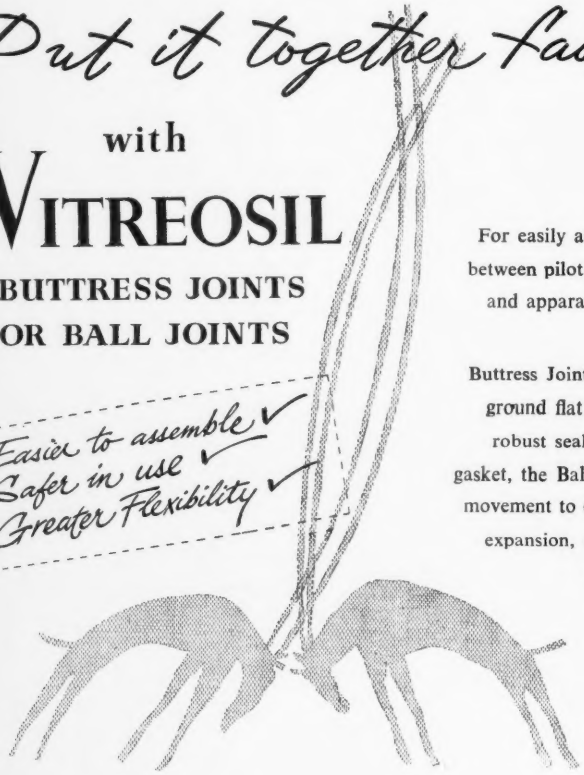
*Put it together faster...*

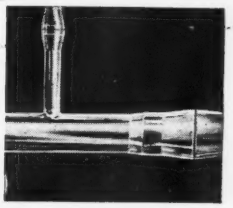
with

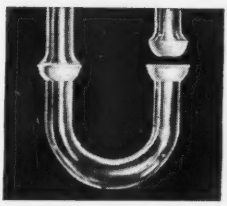
**VITREOSIL**

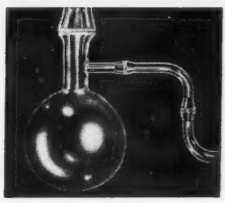
**BUTTRESS JOINTS OR BALL JOINTS**

*Easier to assemble ✓*
*Safer in use ✓*
*Greater Flexibility ✓*









For easily assembled joints between pilot plant pipelines and apparatus these joints are ideal.

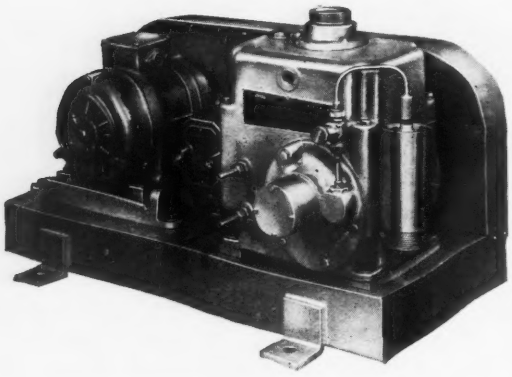
Buttress Joints with smooth ground flat faces ensure a robust seal with a simple gasket, the Ball Joints permit movement to compensate for expansion, contraction, or mis-alignment.

Available separately or fused to tubing or apparatus, both these joints are secured with a simple clamp and are not liable to seize up with such materials as silicon tetrachloride.

TSL

**THE THERMAL SYNDICATE LTD**

P.O. BOX NO. 6, WALLSEND, NORTHUMBERLAND. Tel. Wallsend 6-3242/3.
LONDON: 9, BERKELEY STREET, W.1. Tel. Hyde Park 1711/2.



*Ultimate Vacuum (without gas ballast)* 0.01 *torr**
*Ultimate Vacuum (with gas ballast)* 0.5 *torr*
*Displacement* 900 *l/min* (30 *ft*³*/min*)

* *Torr*=*mm. Hg.* (*B.S.* 2951-1958)

# A New INDUSTRIAL Vacuum pump with LABORATORY Appeal...

Combining the important features of both the industrial types of "SPEEDIVAC" pumps and the laboratory model the 1SC900 is ideal for large scale research, pilot plant and production work—in fact, anywhere where ruggedness and a dependable high vacuum performance are necessary.

Exceptionally quiet and vibration free for its size, this gas ballasted pump is fitted with a patented automatic device to prevent air suck back in the event of accidental stoppage. Mounted in combination with a smaller "SPEEDIVAC" pump and condenser, the 1SC900 will provide a two-stage pump combination capable of handling large quantities of vapour.

## "SPEEDIVAC" HIGH VACUUM ROTARY PUMPS

**EDWARDS HIGH VACUUM LTD., MANOR ROYAL, CRAWLEY, SUSSEX, ENGLAND.** Crawley 150

Q+S+E=NBCo

*... a proven formula*

**QUALITY *plus* SERVICE *plus* ECONOMY**
***equals* NUTRITIONAL BIOCHEMICALS CORPORATION**

- Over 300 Amino Acids
- Over 90 Peptides
- More Than 200 Nucleoproteins, Purines, Pyrimidines
- Miscellaneous Biochemicals
- Vitamins
- Enzymes • Crystalline • Purified
- Growth Factors
- Steroid Hormones
- Biological Salt Mixtures
- Biological Test Materials
- Peptides
- Carbohydrates
- Purified Proteins
- Fatty Acids
- Antibiotics
- Alkaloids
- Glandular Substances

*Three days after your order is received it will be delivered in most parts of Europe.*

Write for New Catalog
July, 1959.
More Than 2,500 Items
Write Dept. 108

**NUTRITIONAL BIOCHEMICALS CORPORATION**

**21010 MILES AVE. • CLEVELAND 28, OHIO**

# NUCLEAR POWER AND ITS DEVELOPMENT

SIR JOHN COCKCROFT is reported to have expressed the opinion on April 26 that in 1966 some 25 per cent of the requirements of the United Kingdom for electricity would be met by nuclear generation, 50 per cent by 1975 and 100 per cent by the end of the century. Questions asked in the House of Commons on June 8 indicate a disposition to allow political and social considerations to over-ride, if not distort, the technical and economic aspects, and there have been other attempts to make the effect on the coal industry the deciding factor in determining the development of nuclear power. The implications of technological change have been ignored, as has the effect of development on the cost of electricity supplied by nuclear power-stations, which Sir Christopher Hinton stressed very strongly in his Axel Axson Johnson Lecture delivered at Stockholm on March 15, 1957.

In his reply on behalf of the Government, the Paymaster-General was emphatic that the programme for the development of nuclear energy in Britain over the next few years was arranged after careful consideration, and it certainly could not be upset on any temporary considerations. An admirable broadsheet, "Prospects for Nuclear Power" (No. 431, March 1959), issued by Political and Economic Planning, is well designed to remove misunderstandings and facilitate an objective approach to this problem, in which it is extremely difficult to separate at all sharply the technical and economic from the political and social elements. Although the broadsheet is particularly concerned with the world position and Britain's export prospects, it includes a lucid discussion of the impact of nuclear power which deserves to be widely read. The brief paragraph on the position in the United Kingdom points out that by 1966, when the dozen or so nuclear power-stations required to supply the target capacity of 5,000–6,000 MW. are in operation, the cost of the electricity generated should be almost competitive with that from other types of power-stations wherever sited; this answers the criticism implied in the questions in the House of Commons.

The broadsheet points out that by 1966 the nuclear power-stations will be supplying a quarter of the electricity used in Britain and doing the work of 18 million tons of coal a year, and that it is estimated that the cost of generating electricity from the largest of the stations now under construction, that at Hinkley Point, will be between 0·59*d*. and 0·66*d*. per kWh., compared with 0·53–0·64*d*. for an up-to-date coal-fired plant. The cost of electricity from the largest station now being built is competitive with that from a high-efficiency coal-fired station built on the same site. Moreover, for the later stages of the present programme, when all new generating plant may be nuclear, Britain is considering types of reactor that will be cheaper to build and more efficient to operate, as well as able to accept re-cycled plutonium to replenish the burnt fuels, and these types should produce electricity more cheaply than conventional plants. Two such reactors are the gas-cooled heavy-water moderated reactor, and the advanced gas-cooled reactor: an experimental version of the latter is already being built at Windscale.

These facts, which were essentially given by Sir Ian Horobin in moving the second reading of the Electricity (Borrowing Powers) Bill on January 20, sufficiently display the tendentious character of the question to which Sir Ian replied in Parliament on June 8. It should also be remembered that the high-temperature gas-cooled reactor is to be studied at Winfrith Heath, and the sodium-cooled fast-breeder reactor at Dounreay, both with the subsequent period in mind when reactors will be needed to take over an increasing proportion of the base load. From the Dounreay reactor, to be in operation this year, will come the data necessary to enable fast reactors using plutonium fuel to be integrated with existing thermal reactors. This will improve the burn-up of natural uranium to somewhere near the theoretical limit. The reactor programme is also supported by large facilities for research and an extensive research programme, while production and fuel-processing facilities are also well developed in Britain, and capacities are sufficient to meet the needs of other countries. Lord Mills, the Minister of Power, stated explicitly in the House of Lords on March 3 that the nuclear-power programme for 1964 onwards had not yet been determined.

This position, however, needs to be set against the general background outlined in the PEP broadsheet, which stresses, too, the dynamic nature of the energy position, both nationally and internationally, and the way in which non-economic influences often determine the most economical way of meeting demands for energy. The great promise of nuclear energy is that, with ample resources of uranium and thorium, and the high energy content of each ton of raw material which makes transport a negligible factor, it will eventually provide all nations with an unlimited and virtually indigenous supply of energy at an economic cost. Although this lies well into the future, nuclear energy should make an immediate though modest contribution, particularly in Europe.

The factors which limit this contribution must be carefully noted in an objective assessment of the situation. First, for technical reasons, nuclear fission reactors are only efficient when employed in large units. The large amounts of electricity produced from each nuclear power-station can in the near future be generated at an economic cost only if they can be used to the maximum. This is only possible where electricity systems are fully developed in a grid-network such as an industrial area provides.

Accordingly, nuclear energy is as yet unlikely to help under-developed countries, nor will it be suitable for some time for the direct production of heat where small units are normal. In Europe, where fossil fuels are expensive, nuclear energy should be competitive within the next ten years for what is called base-load operation, that is, power-stations operating virtually all around the clock feeding electricity into the grid.

The second factor is that small coal is particularly suitable for power-stations, and the proportion of small-coal production is continually growing as more coal is mined by mechanical methods. This coal can only be burnt efficiently in very large plants and its use is thus almost confined to power-stations. Criticism of Britain's nuclear-power programme arises largely from this fact that nuclear energy is suitable for the production of electricity only in the conditions to which the growing quantity of small coal is also suited.

The third factor is that production of electricity at a price competitive with conventional power only means an additional supply of electricity and not the introduction of a cheap new fuel. Electricity has its own particular advantages, and its use in Britain is continually growing ; but it is not, at present costs, an economic substitute for other sources of energy for many purposes. The nuclear-power programme should enable those needs to be met more easily, but it will not obviate the need for other fuels for these purposes. Nevertheless, the development of nuclear energy holds great promise, and Britain's prompt start has given her an early lead in tackling the vast technical problems involved, and only the U.S.S.R. and the United States have programmes at all comparable in size to the British. Moreover, the British power programme, based on the gas-cooled natural-uranium reactor, is the only large-scale programme which is being carried out in a Western country, and the British type of reactor has been proved in operation, having supplied electricity to the grid for some two years.

Nevertheless, the survey in *Planning* of the prospects for British exports of nuclear power-stations and related fuel and equipment leads to the conclusions that the prospects for British firms to export reactors are not so bright as they were once thought to be. Nuclear power is indeed regarded as the most helpful long-term solution of the fuel problem in many European countries, and the principal countries are in a position to exploit nuclear power without too much difficulty. They possess the necessary scientific and technical skills and the capacity to build equipment, and they will therefore want to develop their own nuclear industry as fast as they can. They may buy one or two reactors from other countries, as Italy has done, in order to gain experience of operation and construction ; but after that they will probably do more and more of the work themselves. They are unlikely to set up their own fuel-processing plant, partly because of expense and partly because most European countries have no uranium deposits and are unlikely to obtain uranium without some control against the production of plutonium. France is the one country of Europe which will have a self-contained nuclear industry.

In the Scandinavian countries, and possibly in Spain and Portugal, competition is likely to be fierce, and although the Calder Hall power-station has impressed potential customers with the merits of the British type of reactor, steady and impressive publicity is required. Outside Europe, the first markets —for the next ten years at least—will be in Japan and India, but China is likely to be tied to Russian developments at first. Several countries in Latin America might prove customers for the British type of large power-station reactor.

Whether British or American power reactors are bought—and these are the only countries at present offering to sell power reactors and supporting the offer with a fuel service—the PEP broadsheet suggests that the future of the nuclear reactor export market is closely tied to a successful small reactor. A cheap small reactor similar to that for moderately powered gas-turbine and diesel-powered generating sets could transform areas which are now under-developed. Here, since British efforts have been concentrated on the Calder Hall type, Britain is less favourably placed than the United States to design reactors for special conditions. Without the urgency of Britain's fuel problems, the United States has been able to experiment with a wide variety of types of reactor, and this could prove an important advantage in meeting the future export demand for a small and flexible reactor system. Besides this, the United States is at present the only country that can supply enriched uranium for reactors abroad.

Britain is now taking the preliminary steps to enable her to meet the demand for small reactors, but it is possible that the Soviet Union, although it has not yet been active in export competition, could also have an advantage over Britain in the matter of reactor types. Nevertheless, the broadsheet points out, reactors are not the only product that can be exported. The one part of the British power-reactor which is not manufactured by industry is the fuel element. The rest, including ancillary equipment, such as turbines, generators, handling gear, processing equipment and control instruments, are all the products of industrial firms. Lord Mills, it is true, has directed attention to the more limited opportunities in the near future for new consortia of firms with design teams trained by the Atomic Energy Authority and which would be capable of tendering for the construction of complete nuclear power-stations ; but at the same time, he emphasized the opportunities for the manufacture of small reactors, including research reactors.

Nuclear energy, as the PEP broadsheet puts it, means that there will be an increased demand for those products which British firms can supply as cheaply as any of their competitors ; but the prospects for British exports of nuclear reactors and equipment are not determined solely by the competitive ability of British firms. One dominant factor will be the political agreements that the Government

is able to make. Marketing nuclear reactors is not purely a commercial undertaking, for, as the broadsheet duly notes, international politics are involved, and unless this is clearly understood British chances of building up an export trade will be small.

For this reason alone, it is important that Britain should establish close and satisfactory relations both with the International Atomic Energy Agency and the European Organization for Nuclear Research, and also with Euratom—the European Atomic Energy Community and its six members. A second factor, however, to which *Planning* does not direct attention is that of scientific and technical man-power. Although in the stricter sense this lies outside the scope of the broadsheet, it is probably the ultimate factor on which the prospects for British development of nuclear energy depend. Unless the resources of scientific and technical man-power in Britain are fully developed and effectively used, Britain is unlikely to be able to seize the opportunities that nuclear energy will bring—even to secure all the advantages which its development in Britain might offer to the economy of the country—still less to hold her own in the keen competition forecast in this broadsheet. Nor is this simply a matter of training sufficient scientists and technologists: it is also a matter of seeing that they are wisely used, that our organization and administration of research are adequate and in balance, and that the administrators and statesmen called upon to handle the complex and interlocked technical, economic and social problems which the development of nuclear power will bring are competent to give due weight to all the scientific and technical issues, no less than to the economic or political aspects of the situation.

For some months Euratom experts have been drafting a new and realistic programme for the six member countries, taking account of changing oil and coal prices. Since the PEP broadsheet was issued, the report on this programme has been finished, but owing to disagreement at the top it has been withdrawn and is not to be published. This disagreement has jettisoned Euratom's plans for all but the 1,000-megawatt programme being carried out with American aid, and it is obvious from the broadsheet that most of the chances open to British industry of tendering for foreign nuclear power-stations have likewise disappeared.

Sir Ian Horobin took a far more confident view in replying in an adjournment debate on the nuclear power programme in Britain in the House of Commons on July 1. He confirmed that latest figures gave the cost of electricity from conventional stations as 0·5–0·65*d.* a unit compared with 0·65–0·7*d.* for electricity from nuclear power-stations; but he emphasized that the statement that nuclear energy to-day is 40 per cent more expensive than conventional power was based on a comparison of the cheapest possible coal and the dearest existing nuclear energy. Sir Ian suggested that the real difference is probably more like 15–20 per cent, and he pointed out that in view of the 70 per cent rise in the price of coal over the past ten years it would have been unrealistic to base policy on the assumption that coal would not be 5–7*s.* a ton dearer than it is now. He also thought that the price of uranium will fall when the present contracts expire and that this factor, with a further slight rise in the price of coal, is likely to decrease the margin of 15–17 per cent against nuclear energy, and that the price of generating electricity by nuclear energy is likely to touch that of generating by coal in the late 'sixties.

Sir Ian Horobin insisted that the British nuclear-power programme is a very carefully considered whole, and that it is probable that what may be called the Calder Hall type of reactor has several years of valuable development before it. About 1961 the advanced gas-cooled reactor should become critical, and if this is successful the first commercial type may be in operation about 1965. The work done with the fast breeder reactor now being developed at Dounreay has been very successful and a period of low-power testing at Dounreay is expected to begin this summer. If all goes well, we may hope to have this type in operation by about 1970, and its low capital cost and its place in the balanced programme offer exciting possibilities, particularly in flexibility. By that time a substantial number of stations of the Calder Hall type would be in operation, all producing plutonium. We must bear in mind, Sir Ian said, the possibility of a situation arising when it would be possible to build very much larger stations than originally conceived and that perhaps only about half a dozen more stations would come into the programme. He repeated that we cannot afford to run any risk of completely unbalancing the structure of the industry in Britain by interfering with the present programme. When that programme is complete, further decisions will be required, and although preliminary consideration is being given by the Government to the subject, it is not thought that it is necessary to decide for a further year or two what stations should be built after 1965. It is very important, he said, that everyone should realize that a nuclear-power programme on the scale of that in Great Britain must be a long-term, carefully balanced programme and that it cannot be interrupted in response to short-term considerations. Britain is now the major civil nuclear Power, and the need for thought and care in investment and design are correspondingly great.

## DARWIN WITHOUT MODERN SCIENCE

### Darwin and the Darwinian Revolution

By Dr. Gertrude Himmelfarb. Pp. ix+422. (London: Chatto and Windus, Ltd., 1959.) 42*s.* net.

AN adequate study of Darwin, his scientific achievements, and the result of his work in all the fields of human endeavour which they have affected makes rigorous demands on the competence of whoever attempts to write such a book. Dr. Himmelfarb brings to her task a concentration of interest and of effort, the former of which is passed

on to the reader by the sustained vigour and elegance of her style and the skill with which the book is constructed, while the mastery of her technique hides the laboriousness of the work involved.

She has read practically everything there is to read by Darwin and on Darwin, including manuscripts hitherto unpublished, and has subjected the material to lucid analysis in terms of the history of Darwin's life, experiences, contacts with other persons, researches, results, doubts and beliefs. He is situated in his intellectual environment, his home life, his ailments real or imagined, and his place in history. The social, scientific, religious, political and general public climates of his day are vividly portrayed, and there can seldom have been a work of biography undertaken with such bibliographical care.

But while it would be difficult to exaggerate the excellence of this book as a contribution to the history of the events, ideas and arguments as a result of which Darwin produced his theory of evolution by natural selection, the case is altered when it comes to the evaluation of this theory in terms of the present state of scientific knowledge, because Dr. Himmelfarb's work is imbued with a relentless aversion to natural selection, pursued by means of a skilful and acute dialectic without reference or regard to the results of scientific research during the past fifty years. Indeed, this is directly implied by the statement (p. 366) that "the present status of Darwinism has not altered much since 1860, when Huxley pronounced it to be not an established theory but a tentative hypothesis". When she tries (p. 368) to substantiate such a view to-day by quoting William Bateson, she gives herself away at once.

That the author is not familiar with, or prefers to ignore, the growing edge of scientific knowledge by observation and experiment during the past fifty years appears from statements such as the following (p. 269): "In the experiments of Mendel and de Vries, new species appeared suddenly in the form of mutations". Mendel neither claimed nor obtained any such results. On the contrary, his genius lay in selecting for his experimental material strains which differed only in one or two characters: differences immeasurably inferior to those of specific rank. As for de Vries's 'mutations', it has long been recognized, thanks primarily to the work of T. H. Morgan and his colleagues A. H. Sturtevant, C. Bridges and H. J. Muller, that they are not mutations at all, but the results of a rare method of 'sporting' by permanently heterozygous strains technically known as the 'crossing-over of balanced lethals'.

When the author attributes to neo-Darwinians the statement (p. 270) that "only the smallest mutations could be favourable and that such favourable mutations were in fact so rare a phenomenon that without natural selection not even a fruit-fly, let alone a man, could have developed", and concludes, "Thus it became the very paucity of variations, the very improbability of their concurrence, that was now made to tell in favour of natural selection", she makes the elementary and very outdated mistake of thinking that that mutation is the only supply of variation. It has long been known that recombination of genes is enormously greater as a source of supply of variation than mutation itself, and it produces gradual change.

Dr. Himmelfarb has not grasped the fundamental significance of the work of Sir Ronald Fisher, whom she calls "the mathematician". He showed, first, that natural selection of genes within the gene-complex is a universal phenomenon, which explains why the genes gradually *become* either dominant or recessive. This is how evolution proceeds. Mendelian genetics itself provides evidence of Darwinian selection. He showed, secondly, that selection is so much more powerful than mutation, that no mutation can have the remotest chance of becoming a normal component of a population if there is the slightest degree of adverse selection exerted against it. He showed, thirdly, that as all organisms are tolerably well adapted to the conditions under which they live in their present environment, the vast majority of mutations are bound to be deleterious to the organisms in the conditions under which they arise, and this proves that any attempt to explain evolution by an appeal to causes which might be supposed to impart favourable qualities or directions to mutations through 'inner feelings', '*élan vital*', 'urges', or the transmission of somatic modifications, is killed stone dead at the start. "Every theory of evolution which assumes, as do all the theories alternative to natural selection, that evolutionary changes can be explained by some hypothetical agency capable of controlling the nature of mutations which occur, is involving a cause which demonstrably would not work even if it were known to exist." The fact that wild species in Nature are highly heterozygous shows that mutant genes, subjected to adverse selection when they first arose, remain as recessives in the gene-pool, and function as a reserve which is drawn on when environmental conditions change, and some of these genes then become dominant by selection in the gene-complex, and established in the population. It can also be shown that selection is exerted in favour of the heterozygous state *per se*.

Results such as these should find a place in any modern appraisal of the position of natural selection at the present stage of knowledge; but Dr. Himmelfarb has preferred to say (p. 276) that "Posing as a massive deduction from the evidence, it (natural selection) ends up as an ingenious argument from ignorance". The word "ignorance" is a double-edged weapon when it is used by an author without any indication of awareness of the experimental results obtained by such distinguished scientists as E. B. Ford, C. D. Darlington, P. M. Sheppard, A. J. Cain, W. H. Dowdeswell, H. B. D. Kettlewell or C. H. Waddington, to mention only those working in Great Britain.

The intercalation of quotations from Darwin, labouring in his unavoidable ignorance of the principles of Mendelian genetics, to disparage arguments which have since been substantiated by the results of modern experimentation in natural selection is regrettable. When Darwin wrote a hundred years ago that "we are far too ignorant, in almost every case, to be enabled to assert that any part or organ is so unimportant for the welfare of a species that modification in its structure could not have been slowly accumulated by natural selection", he was quite correct, and it was merely tendentious for Dr. Himmelfarb to say (p. 276) that "three negatives do not normally constitute a positive". When Darwin went on to say that "it would be extremely bold to maintain that no serviceable transitions are possible by which these (electric) organs might have been gradually developed", he was prophetic, as Dr. Lissman's researches have since proved. The initial stages in the evolution of flight in birds, colour-vision, or the whale's adaptation for avoiding caisson-disease, to mention only a few additional striking

cases, have all been shown to be capable of conferring survival value from the inception of the improvement.

The statement (p. 277) that "The eye is obviously of no use at all except in its final, complete form" completely ignores the comparative anatomy, embryology and physiology of the lower chordates, which show how light-sensitive cells in the epidermis have been brought into place, from the surface of the skin into the neural tube and then into the retinal layer of the eye-cup, and are functional at each stage. Dr. Himmelfarb (p. 279) accuses Darwin of invoking the *perfection* of the eye at the same time as he quoted Helmholtz on the inadequacy and imperfection of the eye; but in the "Origin of Species" (World's Classics edition, p. 190), Darwin wrote, "its marvellous yet not absolutely perfect characters". What Darwin claimed was that natural selection confers *improvement*, and in the case of the vertebrate eye this is undeniable to anybody familiar with the visual organs in *Amphioxus*.

The statement that "the persistence without change of any forms over a long period of time is difficult to explain by natural selection" is particularly unfortunate, because so long ago as 1878 T. H. Huxley pointed out that natural selection is the only mechanism that can account both for change and for stability, and as recently as 1952 E. B. Ford supplied the genetic explanation of this by showing that the non-contamination of genes and the rarity of mutation produced stability, while the power of recombination of genes can produce endless variability, under the control of natural selection in each case.

"How can selection, knowing nothing of the end or final process, function when the only test is precisely that end or purpose?" asks Dr. Himmelfarb (p. 277). The experimental results of investigations on the evolution of industrial melanism in moths show how the selective taking by predators of prey ill-adapted to their environment can be seen going on, and the intensity of the selection pressure can be measured with mathematical precision; mimetic resemblances in butterflies are improved and confer survival value in mathematical relation to the prevalence of distasteful models; the method of capture, marking, release and recapture enables the predations by thrushes on snails of different colours to be correlated with the seasonal variation of vegetation, and the longevity of different genetic types of moths to be measured in terms of concrete units of time. That is how selection actually operates.

Astonishingly out of date is the statement (p. 284) that "the entire discussion of sexual selection is anthropomorphic in its basic conception, for whether the coloration of a bird is judged to be either beautiful or monstrous, it is by human standards that the judgment is made". It is now quite clear that the 'beautiful' *and* 'monstrous' colours in question, to which must be added structures, attitudes, and behaviour-patterns used in courtship, function, as Sir Julian Huxley, N. Tinbergen and many others have proved, as stimuli by which a threshold physiological condition in the partner is reached and a release mechanism set in motion. There is no question of any anthropomorphic æsthetic choice.

It is quite correct that many of the examples which Darwin thought conformed to the principle of sexual selection have since been found to owe their origin to other than epigamic causes, such as warning marks, recognitional or threatening defensive characters. But some characters, as those of the male peacock, ruff, or argus pheasant, are good examples of the sexual selection in Darwin's sense, as Sir Julian Huxley showed more than twenty years ago; yet Dr. Himmelfarb concludes (p. 300) that "Sexual selection has all the faults of natural selection and more: the suspicious facility with which it can be made to explain anything and everything; the manipulation of evidence for whatever purposes are convenient; and the invocation of ignorance when all else fails". Here, again, the historical present tense is used, but there is nothing to show that the author does not intend it to apply to the present and that the remarks made represent the actual state of scientific knowledge.

Turning now to another discipline of science, we find Dr. Himmelfarb stating (p. 271) that, "Geology, however, has been notably unforthcoming, and instead of being the chief support of Darwin's theory, it is one of its most serious weaknesses". It is difficult to believe that such a phrase could have been written in 1959 for 1959. She goes on to say that "It might have been expected . . . that in those cases where the geological record is more or less complete . . . we would find . . . closely graduated varieties of species existing at the beginning and at the close of the period. Yet even here we do not find such a graduated series". It can only be concluded that she has not held in her hands the series of ammonites from *Liparoceras* through *Androgynoceras*, *Amaltheus* to *Pleuroceras*, demonstrated by L. F. Spath, nor the series of *Micraster* demonstrated by D. Nichols. As for the series of horses, Dr. Himmelfarb's information is not more recent than that of T. H. Huxley in 1870. Actually the fossil series of ancestors of the horse is now so good that George Gaylord Simpson has been able to measure the time required for the conversion of a species into another and the length of life of a species (two million years in horses). T. S. Westoll had done the same for fish, which evolve at different rates. Simpson was also able to show that the geological record in some places is so good that it is now possible to calculate the degree of variability of fossil species and to prove that it is not correlated with their evolution-rate. This is further evidence that natural selection, not mutation, controls the rate and direction of evolution.

The statement that the geological record is one of the most serious weaknesses of Darwin's theory is ridiculous. Even in his day, the Mesozoic mammals of the Stonesfield Slate had been discovered; and that most beautiful of all known fossils, *Archaeopteryx*, was known, showing such a perfect transition between the reptilian and the avian stages of that line of evolution that it can be regarded as ancestral to all later birds. It also demonstrates the way in which one vertebrate class become converted into another, by piecemeal transformation of bits of the body one by one, a process called mosaic evolution. Other examples of representatives of precursors are *Jamoytius* between the lowest chordates and the fishes, *Ichthyostega* between fishes and amphibia, *Seymouria* between amphibia and reptiles, *Ictidosaurs* between reptiles and mammals, *Australopithecus* between apes and man. All these, and countless others, are a striking vindication of Darwin's views.

The sudden appearance in the geological record of representatives of important groups (such as the gastropods or vertebrates) is no fly in the ointment of Darwin's theory, and it receives a logical explanation from the obligatory rarity of tentative initial types, and from the principle of 'clandestine evolu-

tion' of young stages without hard parts and therefore not preserved as fossils until they became adult in their new state. As for the abrupt appearance of fossils in "the lowest fossiliferous strata", the number of fossils discovered in Pre-Cambrian deposits goes on increasing and now includes algae and fungi in which eight amino-acids could still be recognized although they are 1,700,000,000 years old.

Dr. Himmelfarb states (p. 310) that chemists showed that all the pieces of the 'Piltdown find' revealed the same fluorine content. This was not the case. The earliest estimations by K. P. Oakley and C. R. Hoskins showed fluorine percentages varying from 3·1 to less than 0·1, estimations correct to within a range of ± 0·1. This proved that all the 'specimens' were not Lower Pleistocene. Subsequent estimations by more refined methods showed that the latest of these were not even Upper Pleistocene. But if Dr. Himmelfarb really thinks that the exposure of the Piltdown fraud "leaves the theory (of evolution), after a century of search, without the much desired link", *Proconsul*, *Australopithecus*, *Pithecanthropus* and Neanderthal man are there to bear witness to what Sir Wilfrid Le Gros Clark has demonstrated from them about the so-called 'much desired link'.

If ever the cult of personality should attempt to invade science, it would cease to be science; and if scientists hold Darwin in honour to-day, it is because the evidence, all the evidence, and nothing but the evidence, provided by the observations and experiments of biologists who have undertaken research in this field, has shown that the natural selection of mutant and recombined genes is the mechanism whereby the evolution of plants and animals in Nature has been brought about.

Gavin de Beer

## THE FAITH OF A REALIST

### Blaise Pascal

The Life and Work of a Realist. By Ernest Mortimer. Pp. 240 + 4 plates. (London: Methuen and Co., Ltd., 1959.) 21*s*. net.

MUCH of Pascal's work, and several books about him, are readily available: what is not so easy to obtain is an assessment of his place in history in keeping with the pedestal upon which his fellow-countrymen are nearly unanimous in placing him. Furthermore, to find an answer to the question as to how much he means to us to-day is assuredly a rewarding task. These things the author has done and the result is a notable achievement. Pascal emerges as a character of gigantic intellectual and spiritual stature; weak of body, indomitable of will, and relentless in his quest for truth.

In the present context, we may perhaps leave aside the well-known facts of his mathematical genius, his contacts with great minds like those of Descartes, Fermat and Desargues, and concentrate upon his theory of knowledge (Chapter 11), which developed from the intense strife going on within him, and which burnt itself out as a consuming fire. Pascal was no mere dreamer, but on the contrary passionately concerned with making things work. His technical skill, if he were alive to-day, would lift him to the summit of electronic computing, and to the highest triumphs of cybernetics and servo-mechanisms. In this sense, he was a realist; his faith transcended it, however, as he reached out towards that greater truth only to be found in charity.

It is from some such position as this that we can best approach Mr. Mortimer's treatment of Pascal's theory of knowledge, for it is essentially here that the present volume finds much of its *raison d'être*. Here too is Pascal's message for the world to-day.

The central concept is that of *le cœur*. By this Pascal did not envisage something "cardiac rather than cerebral". He uses the phrase to cover a species of synthesis, a type of thought in which analysis gives place to cognition. It seems as if this came out of a state of mind akin to despair, in that, for example, the propositions of Euclid needed acceptance of something "given" before any progress could be made, and thus real knowledge could never be obtained. In this, he was in effect anticipating Gödel's theorem, and the failure of Hilbert to construct a consistent system of mathematics purely mathematically. But for Pascal, truth is not apprehended by reason alone, which can only yield statistical properties.

Mr. Mortimer is at pains to point out that Pascal did not draw this inference himself; it is nevertheless the gist of his whole argument. Here indeed is a startling preview of twentieth-century science, quantum theory, operationism and all. Nevertheless, the part to be played by *le cœur* remains, and it stands supreme if we are to 'know' the world around us. But what is it, if it is not rational knowledge? Pascal gives his answer—"Le cœur a ses raisons, que la raison ne connait point". Metaphysics may be out of fashion at the moment: it looks, however, as if the faith of a great realist may have elevated such a discipline to a position otherwise unheeded.

As people exclaimed in another setting altogether, "We have seen strange things to-day". The author has written a book modest in compass but great in concept. He has brought Blaise Pascal, his tempests stilled at the last, into the centre of contemporary thought.

F. I. G. Rawlins

## ENZYMES—KINETICS AND CHEMISTRY

### Behavior of Enzyme Systems

An Analysis of Kinetics and Mechanism. By John M. Reiner. Pp. xii + 317. (Minneapolis, Minn.: Burgess Publishing Company, 1959.) 6.50 dollars.

### Proceedings of the International Symposium on Enzyme Chemistry

Tokyo and Kyoto, 1957, organized by the Science Council of Japan under the auspices of the International Union of Biochemistry. (I.U.B. Symposium Series, Vol. 2.) Pp. 541. (Tokyo: Maruzen, 1958.) n.p.

LET Dr. Reiner speak for himself. In his foreword addressed to "Timid Souls" he writes, "The foremost purpose of this book, accordingly, is: To make it possible for anyone to begin the book knowing substantially nothing, and to finish it an expert for all practical purposes". This is a bold ambition, even when restricted to the field of enzyme kinetics. A major adverse criticism of this book is, in the reviewer's opinion, the almost complete lack of reference to

published experimental work ; one has to turn many pages before one can see even the name of an enzyme and there are virtually no numerical data given. Dr. Reiner's ideal reader, the ignorant but intelligent man, but one, nevertheless, longing to learn, would find himself bewildered by reality. Putting these criticisms aside, this book is a serious piece of scholarship, and provides a useful introduction to the theory of enzyme kinetics.

In contrast to Dr. Reiner's solo performance, the Proceedings of the International Symposium on Enzyme Chemistry, relating to a conference which took place in Tokyo and Kyoto in October 1957, contains contributions from 228 authors. Apart from four special lectures delivered by Profs. Chance ("Cytochromes—their Nature and Function in Living Cells"), Engelhardt ("Enzymology and Mechanochemistry of Tissues and Cells"), Lynen ("Phosphatkreislauf und Pasteur-Effekt") and Tamiya ("The Koji, an Important Source of Enzymes in Japan"), the Proceedings are divided into four sections.

The first section covers the mechanisms of enzymatic group transfer, the second, enzyme systems of hydrogen, oxygen and electron transport, the third, the formation of proteins and enzymes, and the fourth relates to enzymes and industry (interpreted to include pharmacology). Many of the contributions are, in effect, short reviews (2–10 pages) of specialized topics, supplemented with what was in 1957 new experimental material. These articles are, in general, of a high standard and are, in the main, very readable.

The title given to the Conference in no way restricted the range of topics ; enzyme chemistry was taken to include any reaction catalysed by enzymes in animals, plants and micro-organisms. Papers dealing with the action of thyroxine on isolated animal mitochondria and the role of chlorophyll in photosynthesis occur in the same section.

The majority of contributions are in English, a few in German and even fewer in French. Almost inevitably some of the papers, from veterans of international conferences, have appeared in substantially the same form before or since.

J. B. CHAPPELL

## CHEMISTRY OF PHOSPHORUS

### Phosphorus and Its Compounds

By John R. Van Wazer. Vol. 1 : Chemistry. Pp. xiii + 954. (New York : Interscience Publishers, Inc. ; London : Interscience Publishers, Ltd., 1958.) 208s.

THIS volume must be unique, for it gives in wide scope and considerable detail an account of the structure, the physical and chemical properties, and the chemical reactions of phosphorus and all its main classes of compounds, both inorganic and organic. It is also outstanding, not only for the wide range of the modern scientific information which is so clearly presented, but also for the historical background of this information : each main topic has a historical introduction, so that, for example, the discussion of the structure of one class of compound may range from a brief review of the theories of a century ago to a more detailed discussion of the most recent evidence supplied by nuclear magnetic resonance spectra.

The first two chapters deal respectively with the nuclear and atomic structure of the phosphorus atom, and with interaction between atoms : the second chapter discusses in detail bond-energies and lengths, dipole moments, polarity of molecules, ionic radii, etc. The following chapters discuss in turn various classes of phosphorus compounds. The detailed information available may be assessed from the 380 pages devoted to phosphoric acid and its compounds, divided into five chapters on condensed phosphates, orthophosphoric acid, chain phosphates, ring and branched phosphates and amorphous phosphates, respectively. This treatment ranges in these five chapters from the physical and chemical properties of phosphate minerals on one flank to those of the nucleic acids on the other. Throughout the book the constant comparison of the properties of the purely inorganic compounds of phosphorus and those of their organic substitution products makes fascinating reading, and may well serve both to widen and to readjust the mental balance with which inorganic and organic chemists have hitherto in their different ways assessed the chemistry of phosphorus.

In the preface, the author makes an eloquent plea that the present division of descriptive chemistry into two parts, organic and inorganic, should now be widened to include a third part, namely, phosphorus chemistry. Many chemists will shrink from this suggestion, but it must be admitted that although phosphorus chemistry contains, on one hand, a number of reactions which can be regarded as normal reactions of inorganic compounds, and on the other hand many reactions typical of organic compounds, there lies in the centre a host of reactions and aspects of behaviour which are peculiar to phosphorus. The same statement might possibly be made of the chemistry of every non-metallic element, but the specific chemistry of phosphorus is, in its range and nature, much greater than that of any other element except carbon. This quality is one of the major factors underlying the vast increase in the academic and technical interest in phosphorus chemistry which has occurred during the past twenty years.

The price of this book may appear high by English standards, but the volume contains an immense amount of information, liberally illustrated by X-ray structure diagrams, phase-rule diagrams, etc., and by a wealth of valuable tabulated material. The final three appendixes list in turn : phosphate minerals (40 pages), giving details of each, based largely on Dana's "System of Mineralogy" ; single bond energies and distances with electronegativity differences ; and thermodynamic data on the compounds of phosphorus.

The book, however, is emphatically not a mere catalogued compilation of facts. In spite of the size of the book, the author has maintained to the end a critical treatment of the material under discussion, and this treatment, combined with the author's pleasant and lucid style, gives the book a personal flavour which heightens the reader's interest throughout the volume.

The publishers are to be congratulated on the general format and printing of the book, and in particular for printing references at the bottom of pages, where they can be immediately noted by the reader, instead of printing them in a vast huddle at the end of each chapter.

The book will be appreciated by all types of chemist, inorganic, organic, physical and phosphoric.

F. G. MANN

## Suggestions to Authors of the Reports of the United States Geological Survey

Fifth edition. Pp. xii+255. (Washington, D.C.: Government Printing Office, 1958.) 1·75 dollars.

IN the eighty years that have passed since the foundation of the United States Geological Survey, more than 3,500 volumes of scientific and technical literature and more than 20,000 different maps have been published under its auspices—an output far surpassing that of any other geological institution in the world. Throughout this long history, continuous efforts have been made to promote lucidity, consistency and uniformity in these publications, and the code of practice established by the Survey for its authors, first published in 1909, has found widespread use outside official circles. The much enlarged fifth edition of this manual outlines the successive literary steps which a geologist-author will normally take from the beginning of an investigation to the final proof-reading of his text, maps and illustrations. It advises on matters of ethics and professional etiquette, enumerates the requirements of a well-prepared manuscript, deals at length with questions of typographical style, and gives a great deal of detailed information on the form and content appropriate to reports of various kinds. More than 50 pages are taken up with sensible advice on composition and expression, forming a sort of "A B C of Plain Words" directed specifically at geologists. Not all the suggestions will be acceptable to British readers, who may be somewhat puzzled by the preferred use of 'geologic' and comparable '-ic' endings in a country with a Geological Survey and a Geological Society. But there is no similar guide produced on the eastern side of the Atlantic; and, with appropriate warnings, the work could profitably become prescribed reading for all post-graduate students of geology, as a brake on the present over-production of 'geologese'.

C. F. Davidson

## Causes de la Répartition des Etres Vivants

Paléogéographie, Biogéographie Dynamique. Par Raymond Furon. (Evolution des Sciences, No. 10.) Pp. 168. (Paris: Masson et Cie., 1958.) 1,000 francs.

THIS book is well described by its author, in his preface, as "ce petit livre de 'morceaux choisis' n'est donc dans mon esprit qu'une esquisse de ce que pourrait être un beau livre qui n'existe pas: un Traité de Biogéographie", for it touches upon almost every aspect of its subject. Unfortunately the touch is too light to permit the suggestion of solutions to the many problems it describes, and this almost inevitably excites, rather than calms, the doubts that haunt most biogeographers as to whether the chaos of facts with which they are confronted can ever be reduced to final order.

Moreover, so vast a subject can be compressed within the limits of a single short book only by the most careful and balanced selection of information, and of the sources from which this comes, and in this respect also the book leaves more than a little to be desired.

With the author's main conclusions, that the present distribution of organisms chiefly reflects the catastrophic consequences of the Pleistocene glaciations, and that to understand the history of the living world its distribution in the Tertiary must be reconstructed, few biogeographers will disagree. They are likely to agree also that palæogeography and palæontology are the keys to this reconstruction, but they are likely to feel some disappointment that an author so well qualified to comment on these particular aspects of the matter does not give a clearer lead as to how they may be more profitably pressed into service.

The great value of the book is as a source of much useful, and not infrequently unusual, factual information, and as such it can be recommended to all who are interested in the distribution of plants and animals.

Ronald Good

## Die Bänderschnecken

Eine Studie zur Evolution der Tiere. Von Prof. Dr. F. A. Schilder und Dr. Maria Schilder. Schluss: Die Bänderschnecken Europas. Pp. iv+93–206. (Jena: Gustav Fischer Verlag, 1957.) Broschiert, 30.30 D.M.

IN this third and final part of their monumental work on polymorphism in the banded snails (*Cepaea*), Prof. F. A. and Dr. Maria Schilder have tried to give an account of the variation in all parts of the ranges of the four species, and to draw some evolutionary conclusions. The booklet contains much useful information but suggests that such a task requires many more workers. The maps summarizing the data may be adequate for some areas of Germany, but it is well known that the proportions of the different colour and banding forms can vary greatly between adjacent colonies; for some large areas far too few colonies have been investigated for any reliance to be placed on mean frequencies from them as truly representative.

The conclusion reached is that the different forms in the polymorphisms have spread out from centres of special abundance. Lamotte's work is quoted as proving that visual selection by predators can never be of importance. The authors seem unaware of published criticisms of both Lamotte's conclusions, which are certainly invalid for Britain, and of their own inferences from their previous work on *Cepaea*. They combine data from colonies for making inferences about selection, although some at least of those colonies are in disturbed habitats and certainly not in genetical equilibrium with their environments. The composition of such colonies in relation to their habitats need give no indication that selection of any sort is acting, even though in fact it may be very strong. The data are given only to the nearest 10 per cent, and are too inaccurate for re-working.

A. J. Cain

## The Sea-Horse and Its Relatives

By Gilbert Whitley and Joyce Allan. Pp. ix+84. (Melbourne: Georgian House, Pty., Ltd., 1958.) 30*s*. net.

APART from a cosy introductory chapter which oozes with unsubstantiated sentimentalities, Whitley and Allan's book will be of value to interested ichthyologists as well as the children for whom it is primarily intended. Besides an account of the lore and legends of sea-horses, there are good descriptions of the structure, behaviour and reproduction of this remarkable fish which Sir J. Arthur Thomson once described as the "most 'kenspeckle' creature of the sea". The systematics of the sea-horse show that about a hundred species have been recorded, and these are distributed over four genera. About half the book is concerned with these and the rest with pipe-fishes, trumpet fishes, flute-mouths, bellows fishes and razor fishes. These, like the sea-horses, are illustrated by some remarkably fine drawings, most of which have been prepared by the authors.

T. H. Hawkins

# RADIATION OBSERVATIONS WITH SATELLITE 1958$_\delta$ OVER AUSTRALIA

By Dr. A. J. HERZ, Dr. K. W. OGILVIE and J. OLLEY

The F.B.S. Falkiner Nuclear Research Laboratory School of Physics*, University of Sydney

AND

R. B. WHITE

Radio Research Board, Commonwealth Scientific and Industrial Research Organization, University Grounds, Sydney

IN June 1958 the School of Physics of the University of Sydney received a cabled request from the Academy of Sciences of the U.S.S.R. asking for help with the recording of signals from *Sputnik III* (1958$\delta$2). As a result of this request, signals from many transits during July and August were recorded with equipment kindly put at our disposal by the Radio Research Board of the Commonwealth Scientific and Industrial Research Organization.

Unfortunately, details of the instrumentation aboard the satellite and of the code used did not reach us until late September 1958, and even now we do not have all the information needed for a complete analysis of the data. We believe, however, that our results are of sufficient interest to be reported at this stage.

## Description of Apparatus

It is now well known[1,2] that among the equipment carried by *Sputnik III* is a scintillation counter. Because of the large size of the crystal (a cylinder of sodium iodide, 40 mm. high and 39 mm. in diameter), the detection efficiency for low-energy photons is high, and fast charged particles give very large pulses, corresponding to the loss of several MeV. or more. In particular, the counter responds with high efficiency to bremsstrahlung photons emitted as the result of the absorption of electrons with energies of about 100 keV. which collide with the sputnik.

A block diagram of the photon counter and its associated telemetering apparatus is shown in Fig. 1, and Fig. 2 gives the pattern of the signals. The second and third pulses carried the information from the scintillation counter. We do not know what information the first pulse carried.

Fig. 1 is largely self-explanatory. The anode current of the photomultiplier is integrated and fed to a bistable circuit which switches relay $A$ at intervals corresponding to a loss of energy in the crystal of $2 \times 10^9$ eV.[1]. The current to the seventh dynode similarly controls relay $C$, which switches at intervals corresponding to an energy loss of $18 \times 10^9$ eV. In addition, the last dynode was connected to a scaler which controlled the switching of relay $B$.

* Also supported by the Nuclear Research Foundation within the University of Sydney.

Table 1. Positions of 1958$\delta$2 during Observations

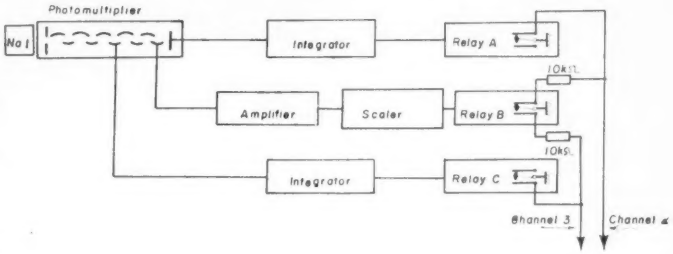
| Epoch | Time (U.T.) | Altitude (km.) | Latitude | Longitude |
|---|---|---|---|---|
| July 19 ·86806 | 2050 | 1,805 | 26·8° S. | 148·9° E. |
| July 19 ·87431 | 2059 | 1,753 | 47·9° S. | 163·7° E. |
| July 23 ·81250 | 1929 | 1,798 | 23·0° S. | 151·8° E. |
| July 23 ·81389 | 1932 | 1,807 | 27·9° S. | 153·7° E. |
| July 30 ·74514 | 1753 | 1,794 | 20·6° S. | 146·5° E. |
| July 30 ·74861 | 1758 | 1,800 | 32·9° S. | 153·5° E. |
| Aug. 4 ·70556 | 1656 | 1,792 | 27·3° S. | 146·9° E. |
| Aug. 4 ·70764 | 1659 | 1,780 | 34·6° S. | 150·9° E. |

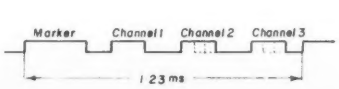
At the time our observations were made, the scaling circuit had ceased to operate so that relay $B$ remained in a fixed position throughout. So channel 2 (the second pulse) transmitted information about the position of the anode-current relay $A$, and channel 3 about the seventh-dynode-current relay. On most of our records the marker and the first-channel pulse are missing as part of the modulating equipment was operating only intermittently, but the data given in this report are all taken from records in which all the pulses are present.

## Positions of the Satellite during Observations

The positions of 1958$\delta$2 used by us were calculated with the aid of the elements of the orbit published by the Smithsonian Institution, and with an orbital period found from a least-squares fit to the transits observed at Sydney.

The results of these calculations are given in Table 1 and displayed in Fig. 3.




Figs. 1 (above) and 2 (below)

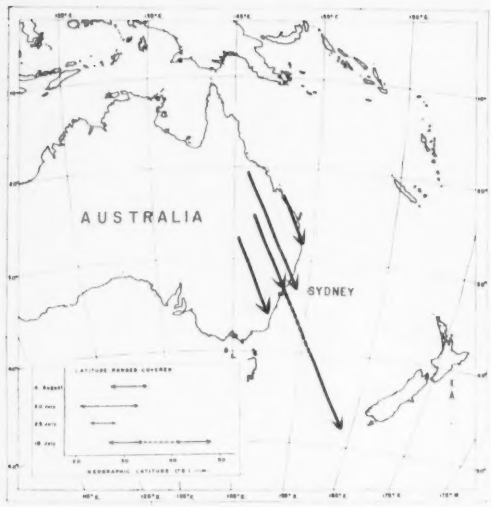



Fig. 3

## Results and Conclusions

The rates of energy loss in the crystal during our five recording periods are plotted as function of time (U.T.) in Fig. 4. Combining these with the positions of the satellite (Fig. 3 and Table 1) we find a minimum of radiation intensity at a geographic latitude of about 35° S. The intensity appears to increase by an order of magnitude when the latitude changes by approximately ten degrees on either side of this minimum. Similar observations over Australia, made with satellite 1958ε (*Explorer IV*), have been reported by Van Allen *et al.*[3], who suggested that these minima correspond to outward-projecting 'horns' in the contours of constant radiation intensity. The minima can presumably be interpreted as the gap between the inner and outer radiation belts which have recently been discovered by means of the Russian artificial planet[4] and by Van Allen and co-workers using the American lunar probes.

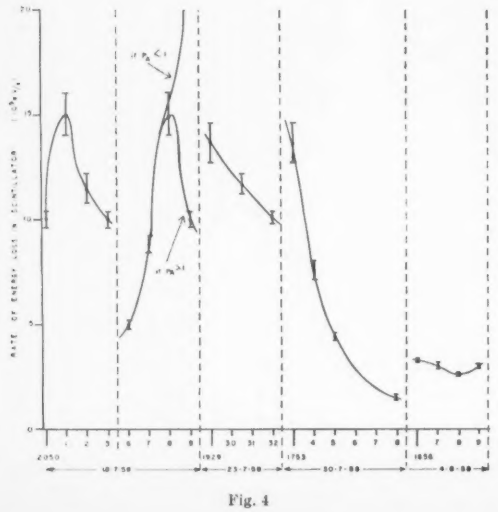


Fig. 4

Our data also suggest a time variation in the radiation intensity: the mean intensity during the first part of the transit of July 19 (see Fig. 4) is about five times the mean for August 4 although the satellite moved through almost exactly the same region in the two cases. We do not know whether the distribution of absorbing matter around the scintillator is such that tumbling of the sputnik could cause such large fluctuations; but, in any event, we do not believe that the fluctuations are so caused since our observations of the signal strength suggest a tumbling period not greater than about 40 sec.—much less than our intervals of observation.

We tentatively conclude that the intensity of radiation in the region observed may show very considerable variations with time. This may be of particular significance as the region in question straddles the gap between the two radiation belts.

Our remaining conclusions concern the ratio of the switching rates of relays $A$ and $C$. It appears to us that the telemetering equipment was not designed to handle the high switching rates, especially of relay $A$, which corresponds to the intensities of radiation encountered by the satellite. The switching rate of relay $A$ almost always overloaded channel 2 during our periods of observation. We were, however, able to obtain adequate data on the rates of loss of energy through channel 3—these are the ones plotted in Fig. 4.

When a large charge passes through the later stages of a photomultiplier, its anode-current response becomes non-linear, and for extremely large flashes of light the current from the dynodes also becomes a non-linear function of the light input. The ratio of the switching rates of relays $A$ and $C$ therefore depends on the average magnitude of the light flashes at low counting rates, and on the average light output from the scintillator at counting rates so high that the interval between the individual events in the crystal is less than the decay time of the light pulses (about 5 μsec. in sodium iodide). According to Vernov *et al.*[1] the ratio of the switching rates of relays $A$ and $C$ is 9 : 1 if the photomultiplier operates in the linear region of its response curve; it will be less if an appreciable number of the pulses is caused by particles which lose large amounts of energy in the crystal, and it will also be less if the number of photons detected in unit time is extremely large.

It was therefore of interest to try to find values for the switching rate of relay $A$, and we were able to do this as we explain in detail below. The results show that the ratio of the switching rates was of the order of 4 : 1, with fluctuations in the range 3·8 : 1 to 6 : 1. Individual results are shown in Table 2; they suggest that the ratio decreased with time. As we have no information about the response curves of the apparatus we cannot draw conclusions about the nature of the radiation from this.

Table 2. The Ratio $p_C/p_A$

| Epoch | Time (U.T.) | $p_C/p_A$ |
|---|---|---|
| July 19 ·86806 to July 19 ·87431 | 2050 to 2059 | 5·0 ± 0·3 |
| July 23 ·81250 to July 23 ·81389 | 1929 to 1932 | 4·4 ± 0·1 |
| July 30 ·74514 to July 30 ·74861 | 1753 to 1758 | 3·9 ± 0·2 |
| Aug. 4 ·70556 to Aug. 4 ·70764 | 1656 to 1659 | 3·0 ± 0·2 |

## Recording Method

The receiving aerials were two horizontal dipoles placed at right angles to each other. Each dipole

Fig. 5

fed a communications receiver, and the rectified signals from the second detectors were further amplified by two separate d.c. amplifiers. The two outputs were then summed by a resistive mixing network and the resultant was applied to the deflecting plates of a cathode-ray tube. The spot was photographed on 35-mm. film moving perpendicular to the direction of deflexion at a speed of 0·6 in./sec. (1·52 cm./sec.).

## Characteristics of the Signal

A sample of one of our records, showing complete pulse trains with marker pulses and first pulses, is given in Fig. 5. The pulse-lengths were measured with the aid of a travelling microscope. A sample of a pulse-length distribution (for the transit of July 23) is shown in Fig. 6.

The various combinations of positions of relays *A*, *B* and *C* lead to pulses of nominal lengths 50, 100 and 150 μsec.[1] which we designated 'short', 'medium' and 'long'. As the position of relay *B* was fixed, the second-channel pulses were either long or short, depending on the position of relay *A* only, and the channel-3 pulses were of either short or medium length. It is clear from Fig. 6 that in both channels there occurred a number of intermediate-length pulses: these were caused by the switching of a relay while a pulse is being transmitted.

With the aid of time markers recorded on the film we were able to measure the pulse-lengths. The results are given in Table 3.

Table 3. Lengths of Pulses and Pulse Trains

| | Nominal value* (m.sec.) | Measured (m.sec.) |
|---|---|---|
| Short pulses | 50 | 69 ± 8 |
| Medium pulses | 100 | 115 ± 8 |
| Long pulses | 150 | 161 ± 10 |
| Pulse train including marker | 1,230 | 1,210 ± 30 |

* Vernov *et al.* (ref. 1).

## Analysis of the Records

In the system of telemetering used, the positions of the relays are sampled at regular intervals of 1·23 sec. (the cycle-length). A change in the length of the appropriate pulse occurs whenever the number of switchings during the preceding cycle was odd; no change is found when the number of switchings was even. If a relay is switched during transmission of the pulse the length of which it controls, a pulse of intermediate length occurs.

If the relay is switched at intervals which are large compared with the length of the sampling cycle, every switching results in a change of pulse-length. Common sense suggests that the converse is also true: that changes in pulse-length at intervals large compared with the cycle-length denote an equal

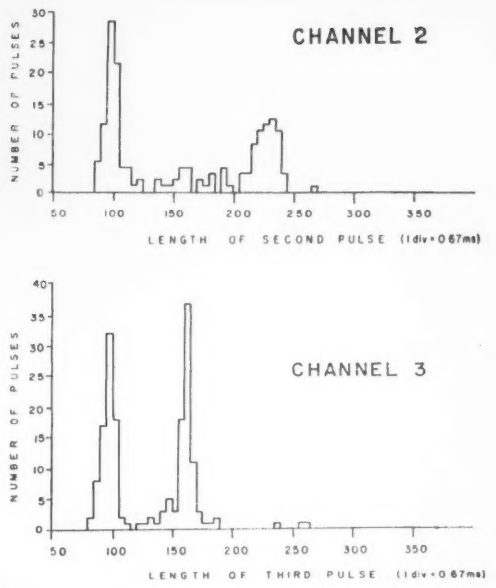



Fig. 6

number of switchings, each of which occurred during the cycle immediately preceding the change. Although not strictly true, as we shall see below, this turns out to be almost always correct.

For more detailed analysis we plot (Fig. 7) the interval between pulse-length changes as a function of the interval between switchings of the relay. It is clear that if the interval between switchings were really constant, and if there were no additional information, we should not be able to deduce a unique value of the switching frequency from the observations of changes in pulse-length. Fortunately, however, the intervals fluctuate, and we have other data as well.

The published information shows that if the photomultiplier is working in the linear region of its anode-current characteristic, the ratio between the switching rates of relays *A* and *C* is 9 : 1. Overloading cannot increase this ratio, so that we accept only those pairs of switching rates which have ratios of 9 : 1 or less. Secondly, as Fig. 7 shows, the lengths of the intervals between changes are very sensitive to changes in the switching rate when there is more

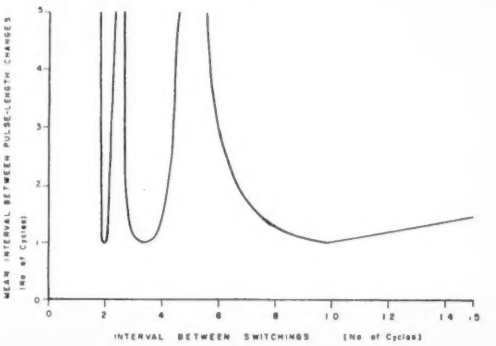



Fig. 7

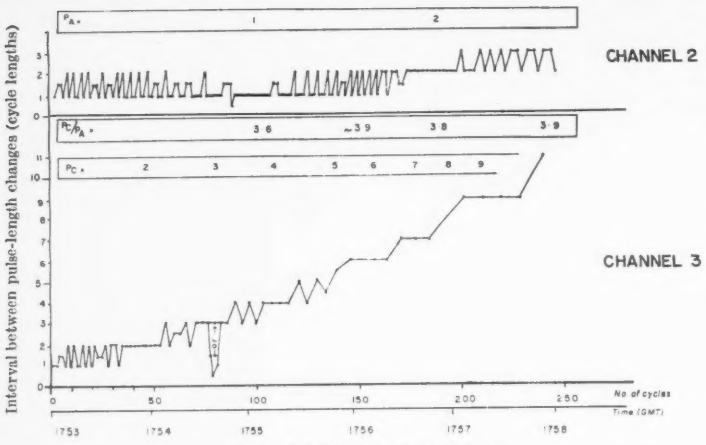



Fig. 8. July 30, 1958. 1753–58 G.M.T.

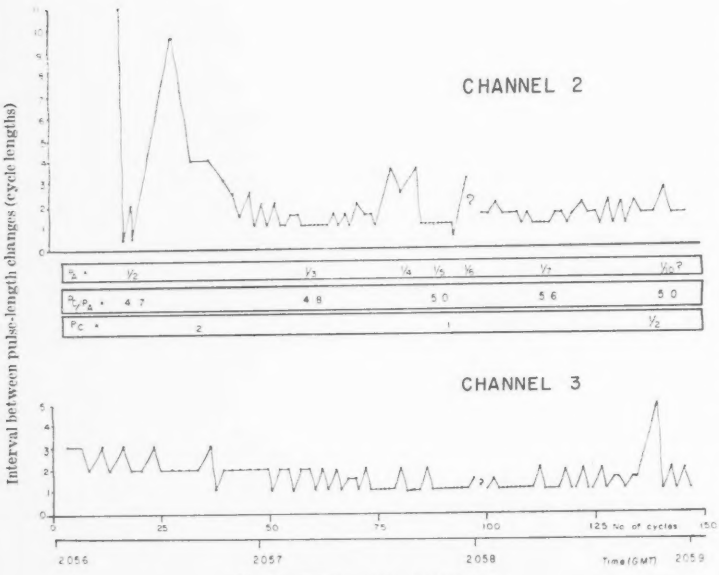



Fig. 9. July 19, 1958. 2056–59 G.M.T.

than one switching per cycle. In the presence of fluctuations it is thus quite unlikely for high switching rates to give rise to long successions of pulses of equal size, that is, to consistently long intervals between changes in pulse-length. We conclude that if the intervals between pulse-length changes are consistently longer than the sampling cycle, the frequency of the changes is equal to the switching frequency of the relay concerned. During most of our observation time the less-sensitive channel 3 operated in this way so that we could deduce the radiation intensity directly from the frequency of pulse-length changes in it.

With one exception, at 1758 U.T. on July 30 (see Fig. 4), the intervals between the switchings of relay $A$ in channel 2 were always less than the length of the sampling cycle. The intervals between pulse-length changes thus rarely exceeded two cycles, and we could not obtain information about the rate of switching from the average magnitude of these intervals alone. However, for the reasons discussed earlier we felt it worth while to try to estimate the switching frequency of relay $A$.

The possible values of the interval $p$ between switchings can be obtained from Fig. 7, and the smaller values of the intervals $p_A$ (between the switchings of relay $A$) can usually be eliminated because of the requirements that the ratio $p_C/p_A$ cannot be greater than 9 : 1. In most cases we were still left with several possible values of $p_A$ from which a choice had to be made using the patterns of pulse sizes in both channels.

Two additional pieces of information helped us here. First, the ratio of the switching rates is not likely to show violent discontinuous changes. Secondly, in spite of fluctuations, there will be a brief series of equal-width pulses (no changes) whenever the number of switchings per cycle is nearly equal to an even integer ($p = 1/n$ cycle lengths where $n$ is even). Whenever there is an odd number of switchings per cycle there will be a series of pulse-length changes every cycle. This effect, though not very pronounced, is clearly observable.

In our analysis we used a graphical method of display, of which we show examples in Figs. 8 and 9. Along the horizontal axis we plot the times of occurrence of changes in pulse-length, while the ordinate gives for every change the time elapsed since the immediately preceding one. We also indicate on the diagrams our estimated values of $p_A$ and $p_C$ and of the ratio $p_C/p_A$. The intervals $p$ are measured in units of one cycle-length.

Fig. 8 shows the record corresponding to our lowest detected radiation intensity: the only case when we found $p_A$ to be undoubtedly greater than unity. It can be seen clearly that $p_C/p_A$ is approximately equal to 3·8—much less than the nominal value 9.

Fig. 9 is a display of data from an observation period during which the radiation intensity was high and changing rapidly. According to our estimates, $p_A$ varied from approximately 1/2 cycle to 1/10 cycle during the time covered by the diagram, and the record shows particularly clearly the peaks in the plot associated with even integral values of $1/p_A$, and the runs of pulse-length changes every cycle which occur when $1/p_A$ is odd.

We should like to thank Prof. H. Messel for the excellent laboratory facilities made available to us, and Dr. G. H. Munro, the officer in charge of the Sydney Section of the Radio Research Board, for making possible the recording of the sputnik transmissions. To Academician L. I. Sedov we are grateful for an informative discussion during his recent visit to Australia, and for the reprints he presented to us. We are much indebted to Miss Xenie Federoff for the careful and conscientious way in which she carried out the tedious work of reading the records. One of us (J. O.) would like to thank the Commonwealth Scientific and Industrial Research Organization for the award of a studentship.

[1] Vernov, S. N., Vakulov, P. V., Gorchakov, Ye. V., Logachev, I., and Chudakov, A. Ye. (in Russian), "Collection of Papers on Sputnik Results", **2** (Moscow: U.S.S.R. Academy of Sciences, 1958).

[2] Chudakov, A. Ye. (privately circulated information).

[3] Van Allen, J. A., McIlwain, C. E., and Ludwig, G. H., *J. Geophys Res.*, **64**, 371 (1959).

[4] Vernov, S. N., and Chudakov, A. Ye. (privately circulated preprint).

# ROBERT HOOKE AND BOYLE'S AIR PUMP

By Dr. H. D. TURNER
University of Sheffield

IT is just three hundred years since Robert Hooke built Boyle's air pump. This event has been described by Gunther[1] as "the most important research ever brought to a successful issue in Oxford"; and it is perhaps appropriate to mark the tercentenary by examining the truth of this statement and, incidentally, by somewhat belatedly giving Hooke the acknowledgment which is his due for his share in the enterprise.

Robert Hooke, who was born on July 18, 1635, at Freshwater in the Isle of Wight, was the son of the local curate. He was a sickly child and never robust, but he gave early evidence of that mechanical aptitude which led to his being described as "certainly the greatest mechanick this day in the world"[2]. After the death of his father, Hooke went, at the age of thirteen, to Westminster School; and in 1653 he went to Christ Church, Oxford, as a chorister, being admitted to the degree of Master of Arts in 1663. During the latter part of his stay in Oxford, Hooke was employed as a laboratory assistant by Boyle, and in 1659 he built the air pump shown in Fig. 1.

At this time Boyle was very much interested in the mechanical properties of the air, and very dissatisfied with the behaviour of the air pump then available, that due to Otto von Guericke. In 1660 Boyle wrote his famous treatise on the "Spring of the Air"[3]. In this book, which was dedicated to his nephew, Lord Dungarvan, he says:

"As few inventions happen to be at first so compleat, as not to be either blemished with some deficiencies needful to be remedied or otherwise capable of improvement: so when the Engine we have comes to be more attentively considered, there will appear two very considerable things to be desired in it.

"For first, the *Wind Pump* (as somebody not improperly calls it) is so contriv'd that to evacuate the Vessel, there is required the continual labour of two strong men for divers hours. And next, (which is an imperfection of much greater moment) the Receiver or Glass to be employ'd, consisting of one entire and uninterrupted Globe and Neck of Glass; the whole Engine is so made, that things cannot be convey'd into it, whereon to try Experiments. So that there seems but little (if anything) more to be expected from it, than those very few *Phaenomena* that have been already observed by the Author and recorded by SCHOTTUS".

Boyle then goes on to say that he asked Hooke to contrive a more effective air pump.

"Wherefore to remedy these Inconveniences, I put both Mr. G(RATORIX) and R. HOOK (who hath also the honour to be known to your Lordship, and was with me when I had these things under consideration) to contrive some Air Pump, that might not like the other, need to be kept under water (which on divers occasions is convenient) and might be more easily managed. And after an unsuccessful tryal or two of ways propos'd by others, the last named Person fitted me with a Pump, anon to be

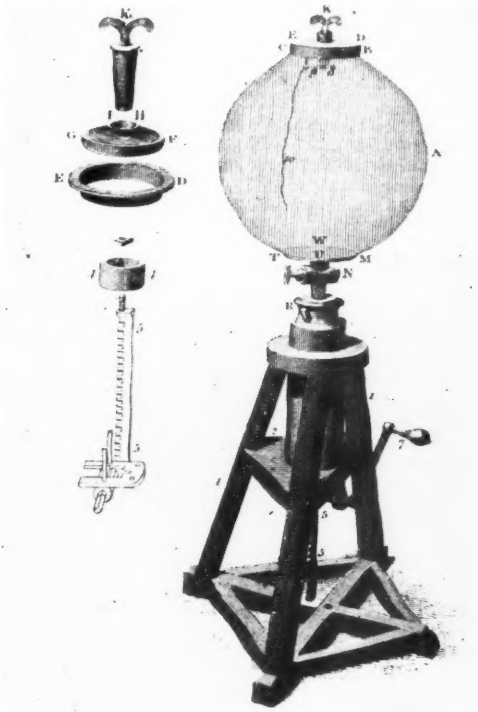


Fig. 1. Boyle's air pump, built for him by Robert Hooke in 1659. This was a notable advance on O. von Guericke's pump since the evacuated enclosure was easily accessible and the ratchet system enabled one man to operate the pump without undue effort. Hooke later built a double-acting pump which was even more effective and flexible in operation

described. And thus the first imperfection of the German Engine, was in good measure, though not perfectly remedied".

The cylinder of the pump was bored in London; but the rest of the machine was constructed by Hooke in Oxford. The technical difficulties were evidently enormous. Boyle goes on to say:

"Your Lordship will, perhaps, think that I have been unnecessarily prolix in this first part of my Discourse. But if you had seen how many unexpected difficulties *we* found to keep out the external Air, even for a little while, when some considerable part of the internal had been suck'd out, you would peradventure allow that I might have set down more circumstances than I have, without setting down any, whose knowledge, he that shall try the experiment, may not have need of".

Once an efficient air pump was available, there were many experiments which could be performed. One of the first investigations carried out by Boyle was into the relationship between the pressure and volume of a gas.

This led to the enunciation of 'Boyle's Law' or 'Boyle and Mariotte's Law' after its confirmation by Mariotte in 1676. Although Boyle does not explicitly name Hooke as his assistant and collaborator in this work, Gunther[4] believes that he was. At this time Boyle was suffering from weakness of eyesight; and he also complained of a lack of skill in geometry which made him "both unwilling and unfit to engage in any Study where the conversing with Mathematical Schemes is necessary". The suggestion is that Hooke, a skilled experimenter and a very able geometer, was, in fact, mainly responsible for the enunciation and proof of Boyle's Law.

Hooke himself carried out many experiments with the air pump. In 1662, having been released by Boyle, he went to London as curator of experiments to the newly formed Royal Society. In this capacity he had to demonstrate different experiments two or three times a week for the delectation of the Fellows; and in many of these he used either the air pump, or the condensing engine, a compressor which he built in 1662–23. For example, in April 1663 we find Hooke experimenting with water freed of air which, according to Huygens, did not subside in a Torricellian tube; and later, air was removed from above and within water containing various fish to see which would die soonest.

This work with fish undoubtedly stimulated an interest in the general problems of respiration and combustion, and we may suspect that this was not unconnected with the interest that the Royal Society was then taking in the problems of diving. In 1664 we find Hooke giving an account of an experiment with two birds, one of which was kept in compressed air and the other in air at ordinary atmospheric pressure. He also constructed a container, large enough to hold a man, which could be partially evacuated by the air pump. Hooke experimented on himself in this device, thus anticipating modern investigations into human behaviour under reduced atmospheric pressure. He tells us that when a quarter of the initial air had been extracted he was able to endure for "somewhat above a quarter of an hour without any other inconvenience than feeling some pain in his ears, and finding himself deaf". During this experiment Hooke took a lighted candle into the container and discovered that it was extinguished long before he experienced discomfort. Other experiments carried out at this time involved the development of an air gun, the measurement of air pressure and experiments with diving bells; but the most important aspect of this period of experimentation is undoubtedly the insight which the use of the air pump gave Hooke into the mechanism of combustion and respiration. The culmination of this work was, in fact, the publication by Hooke in 1664, in "Micrographia", of his theory of combustion[5].

In 1680 Denys Papin, who invented the 'digester' (the prototype of the modern pressure cooker), joined Hooke as his assistant. He later developed a 'steam pump' in which water was forced, by expanding steam, into a container from which it was ejected, under pressure, on to the paddles of a water-wheel; and this has been regarded by some French authors as the first steam engine.

Papin's claims in this direction, however, must, one feels, rest on his contributions to the development of the atmospheric engine. For some time on the Continent there had been interest in the possibility of producing power by using the pressure of the atmosphere to force a piston along an evacuated cylinder. Various ways of producing the vacuum had been tried. Guericke[6] had described an experiment in which a cylinder had been evacuated by using his air pump; Huygens[7] had blown the air out of a cylinder by the detonation of a small charge of gunpowder; and Papin[8] had used the apparatus shown in Fig. 2 to produce a vacuum by the condensation of steam. Like many other members of the Royal Society at this time, Papin was aware of the enormous problem of clearing flooded mines

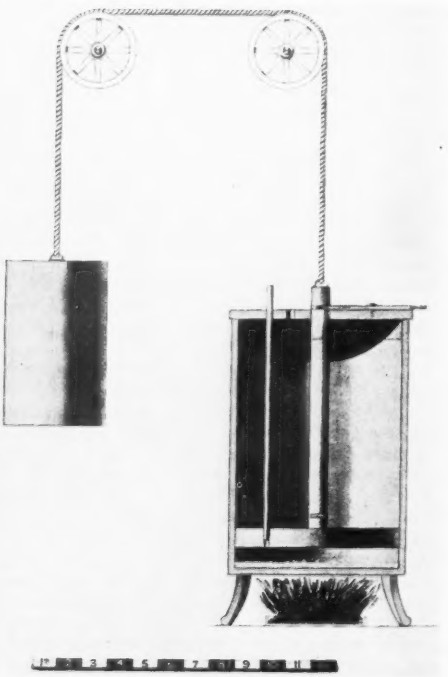


Fig. 2. Papin's cylinder and piston device, 1690. (*Crown copyright. From an exhibit in the Science Museum, South Kensington*)
Steam generated by heat from the fire allowed the piston to rise to the top of the cylinder, where it was locked in position by the movable rod. The device was removed from the fire; the steam condensed by pouring cold water on the cylinder; and on releasing the piston it was forced down by air pressure, thus raising the counterbalanced weight

which were frequently distant from sources of water power. In 1685 he proposed a scheme for the transmission of power over considerable distances. A water-wheel was to drive two large air pumps; the air contained in pipes attached to these would be alternately rarefied and compressed; at the mine the suction and pressure would alternately lift water from the mine and then expel it. In 1688 he proposed a modification of this in which a conveying pipe was to be evacuated by a water-driven air pump; at the mine the conveyance pipe was connected by branched pipes and valves to two vertical cylinders fitted with pistons; these were connected by ropes to an axle carrying a winding wheel around which was wound another rope carrying buckets at each end. As each cylinder was evacuated in turn, external air pressure would force its piston down, thus turning the wheel first clockwise, then anticlockwise, and hence the buckets would be alternately raised and lowered.

Hooke's influence on these projects and his contribution to the ultimate development of the atmospheric engine is now, unfortunately, undocumented. Our only evidence of his interest and suggestions comes from references made by Dr. Robison[9] of Edinburgh. Among the latter's papers after his death was found a "List of Dr. Hooke's Inventions" which contained the following sparse note, "1678 proposed a Steam Engine on Newcomen's principle", which implies that he had anticipated later developments in this field. Robison also claimed to have seen, among the Royal Society's collection of Hooke's papers, memoranda of a letter addressed to Thomas Newcomen, of Dartmouth; these memoranda were, however, not in the possession of the Royal Society in 1880. According to Robison, Newcomen and John Cawley, a glazier, were anxious to make an engine on the lines suggested by Papin in 1688. Newcomen was in touch with Hooke and wrote to ask his advice. Hooke had already criticized both Papin's projects on the ground that the great compressibility of the air in the conveyance pipe would result in negligible effects at the mine unless the water-driven pumps had inordinately long strokes. In replying to Newcomen, Hooke said, "Could he [Papin] make a *speedy vacuum* under your second cylinder, your work is done"[10].

This suggestion was taken up by Newcomen and Cawley, who probably knew of Papin's piston and cylinder experiment and who certainly knew of the methods employed by Savery in operating his pumping machine, 'The Miner's Friend'. According to Stuart[10], "They, therefore, made the experiment of introducing *steam* under a *piston moving in a cylinder, and formed a vacuum by condensing the steam by an affusion of cold water, on the outside of the steam vessel; and the weight of the atmosphere pressed the piston to the bottom of the cylinder*".

This method of producing a vacuum by the condensation of steam had been patented by Savery. An agreement was therefore entered into between Savery, Newcomen and Cawley, and they were all associated in the grant of a monopoly for the atmospheric engine which was made in 1705.

The construction by Hooke of the first efficient air pump undoubtedly exercised a profound effect on the development of science and technology in the second half of the seventeenth century. Not only did the use of this machine lead to an understanding of the physical properties of gases and to a theory of combustion which is strikingly similar to the modern theory; but also it focused attention on the properties of systems of pistons and cylinders and the pressure of the atmosphere. This was certainly important in providing the climate of opinion in which the atmospheric engine could develop; and as we have seen, there is reason to believe that in this development, too, Hooke played a decisive part.


[1] Gunther, R. T., "Early Science in Oxford", **6**, 8 (Oxford, 1930).
[2] Gunther, R. T., "Early Science in Oxford", **6**, 6 (Oxford, 1930).
[3] Boyle, R., "New Experiments Physico-mechanical touching the Spring of the Air and its Effects, Made for the most part in a New Pneumatical Engine" (Oxford, 1660).
[4] Gunther, R. T., "Early Science in Oxford", **6**, 73 (Oxford, 1930).
[5] Hooke, R., "Micrographia", 103 (London, 1664).
[6] Guericke, O. von, "Experimenta nova" (1672).
[7] Huygens, C., "Œuvres Completes", **6**, **22**, **7** (The Hague, 1895).
[8] Papin, D., "Fasciculus dissertationium de novis uibusdam machinis" (Marburg, 1695).
[9] Robison, J., article on The Steam Engine, "Encyclopædia Britannica", Third Edition. "A System of Mechanical Philosophy with Notes" by D. Brewster (Edinburgh, 1822).
[10] Stuart, R., "A Descriptive History of the Steam Engine", 59 (London, 1824).


Information about Hooke's experiments on respiration and combustion and with the air pump has been taken from "Early Science in Oxford", vols. 6 and 7.

# CANCER AND THE RESPIRATORY GRANA


By Prof. CARL C. LINDEGREN

Biological Research Laboratory, Southern Illinois University, Carbondale, Illinois


WARBURG'S[1] theory of the origin of cancer proposes that tissue specificity can be achieved only by the synthesis of substances which make tissues specifically different through oxidative metabolism in the grana which carry the oxidative enzymes. Accordingly, the loss of tissue specificity begins with the loss of oxidative capacity through injury to the grana. Since a source of energy is essential, the cell can only survive the loss of its oxidative apparatus if the oxidative apparatus is replaced by the adaptive development of the fermentative apparatus. The loss of oxidative capacity is not reflected by an immediate loss of tissue specificity; but when the injured cell divides and the daughter cells begin to grow, they do not achieve tissue specificity because the energy produced by fermentative metabolism cannot produce it. Warburg has focused attention on the oxidative grana of the cell; mutation, the carcinogenetic poisons, viruses, mechanical irritations, anaerobiosis, radiation and all other 'indirect' causes of cancer and influences, similar to hormonal control, are assumed to affect the autonomous grana and thus to affect tissue specificity only through their action on the grana. Warburg has unified all ideas concerning the origin of cancer into a single concept involving material organelles capable of

observation and metabolic investigation, thus making it possible to bring the phenomenon under test and observation. In this respect, the theory is (as Warburg says) the only explanation of the origin of cancer cells which can be "metabolically specified". The essential aspects of the theory are summarized by Warburg: "Cancer cells originate from normal body cells in *two* phases. The first phase is the irreversible injuring of respiration . . . there is only one common cause into which all other causes of cancer merge, the irreversible injuring of respiration.

"The irreversible injuring of respiration is followed, as the second phase of cancer formation, by a long struggle for existence by the injured cells to maintain their structure, in which a part of the cells perish from lack of energy, while another part succeed in replacing the irretrievably lost respiration energy by fermentation energy. Because of the morphological inferiority of fermentation energy, the highly-differentiated body cells are converted by this into undifferentiated cells that grow wildly—the cancer cells."

Although Warburg's theory concerns the problem of dedifferentiation, it does not explain how the cell became differentiated. It has been pointed out by others that the cancer cell is not dedifferentiated back to the embryonic level but always retains some characteristics which make it identifiable with regard to origin. Thus the loss of tissue specificity does not involve complete but only partial 'dedifferentiation'.

Differentiation is also achieved in organisms in which defined cells do not exist, since the Ascomycetes, which are cœncytial, are highly differentiated. Even though the nuclei flow freely through the false septa throughout the thallus, differentiation into mycelium, conidiophore and conidium is achieved in circumstances so fluid that no specific cell can be identified. This differentiation requires, like all other differentiation, the activity of many genes, since many nonconidial forms of *Neurospora* are known each of which is the phenotype of a different recessive allele. It may be inferred that differentiation is effected differently in cellular and noncellular organisms.

Although we all speak freely about 'yeast cells', it should be pointed out that phylogenetically yeasts are also noncellular organisms which graduate from mycelial or cœncytial forms to budding varieties all of which seem to be relatively closely related. The so-called yeast 'cell' and its bud is simply a cœncytial form which partitions single nucleate buds with a surrounding wall, much as *Neurospora* forms microconidia. Although this might seem like splitting hairs, it is a vital point in cell theory. The 'cell' that Warburg means is the cell of a multicellular organism.

A dominant scientific theory remains the dominant theory until it is replaced by a subsequent theory, because scientific effort can only be carried out under the ægis of a theory which restricts the activities and directs the efforts toward the examination of fundamental principles. Warburg's theory is to-day the dominant theory; by unifying the concept Warburg has made all other ideas concerning the origin of cancer subsidiary to his. In the absence of an alternative theory, one does not reject a theory by indicating extreme dissatisfaction with it (for example, Weinhouse[2]), since no scientific activity is conceivable without a guiding theory. The most adverse criticism notwithstanding implies acceptance *ipso facto*.

The first step in criticism of the theory is the specification of the assumptions upon which it is based. Warburg has assumed: (1) that the ascites tumour cell is a typical cancer cell; (2) that differentiation and tissue specificity in cellular organisms are achieved by oxidative metabolism of the grana (the respiratory grana have functions other than tissue differentiation, since they play an active part in the metabolism of yeasts); (3) that the grana are autonomous; and, therefore, (4) that the oxidative apparatus cannot be synthesized *de novo* (recent, unpublished, experiments in this laboratory have revealed that respiratory-deficient yeast cells, which never recover respiratory capacity spontaneously, can in certain unique conditions become respiratory-sufficient by exposure to yeast homogenates); (5) that cells which have lost oxidative capacity may obtain their energy by fermentation; (6) that division of these fermentative cells leads to the loss of tissue specificity, since they cannot obtain the oxidative energy essential to differentiation; (7) that such cells can neither fit into the tissue to perform the tissue function nor be restricted by the agents which restrict the differentiated cell and, therefore, form tumours; (8) that oxidative capacity can be injured (*a*) by oxidation of narcotics on the grana, (*b*) by direct action of specific poisons, (*c*) by anaerobiosis, (*d*) by radiation and (*e*) by various other items which are carcinogenetic. Research aimed at understanding cancer (as distinguished from applied research aimed at methods relieving the symptoms, study of surgical procedures, discovery of drugs for destroying cancer by large-scale canvasses, etc.) requires an investigation of the validity of these assumptions and a reformulation of theory depending on the results of such investigations.

Warburg's theory presupposes that the aerobic apparatus is a recent phylogenetical achievement. It is clearly a late, almost certainly the last, great advance made by the free cell in evolution, since it could only occur after photosynthesis had made oxygen available. Its recent integration into the cell probably accounts both for its autonomy and its vulnerability. (The respiratory grana and the chloroplasts are both autonomous structures which were obviously integrated into the cell recently, presumably before multicellular organisms evolved. It is reasonable to suppose that the chloroplast was the original symbiont added to the cell—or to the cœncyte—and that it later evolved into the respiratory granum with the advent of oxygen.) Since the autonomous aerobic apparatus seems universal, it may have been perfected when cells existed only as free cells, before the advent of the differentiated multicellular Metaphyta and Metazoa. The adaptive enzymic fermentation in yeasts, which is normally achieved by oxidative metabolism, may also be achieved by fermentative metabolism, albeit more slowly and less efficiently. Warburg might say that enzymic adaptation in single-celled micro-organisms is a 'cytoplasmic' activity and thus different from 'true' tissue specificity in multicellular organisms. On this theory, true tissue specificity may have appeared after the advent of oxygen.

A striking difference between yeast cells and mammalian cells lies in their means of obtaining energy. Yeast cells, unlike mammalian cells, have two separate fully functional systems for supplying adenosine triphosphate—an oxidative and a fermentative system. Yeasts can grow oxidatively or fermentatively without previous preparation, unlike the mammalian cells which can adjust themselves to a fermentative mode of existence only (according to Warburg)

by slow degrees. In this sense, mammalian cells are almost obligate aerobes. No obligately aerobic yeasts are known since the anaerobic metabolism in yeasts is always available as a source of energy. In some yeasts (in which the aerobic pathway has been lost) the anaerobic pathway is the only source of adenosine triphosphate. Yeasts are markedly different from the filamentous fungi, like *Neurospora*, in which it is difficult to demonstrate the fermentative pathway and which resemble mammalian tissue in growing only poorly, if at all, under anaerobic conditions.

This discussion suggests that the yeast cell can provide a fruitful research object for study of the sensitivity of the respiratory apparatus and thus a guide in the evaluation of carcinogens. Many kinds of poisons have been tested with this view in mind. An interesting poison which was introduced to us by Dr. Seymour Hutner is propamidine isethionate. It is widely used in the tropics as a specific for kala-azar. It is thus important to know whether or not it may be carcinogenetic. Our present indications are that it destroys the respiratory apparatus too completely, too rapidly and too specifically to be a carcinogen—if the data from yeast cells are transferable to humans. When yeast cells are smeared on the surface of an agar plate, and a paper filter pad saturated with a solution of 1750 p.p.m. of propamidine isethionate is placed in the middle of the plate, a zone (several cm. wide) appears around the disk in which the yeast cells grow abundantly but in which none of the cells is capable of utilizing oxygen. Since the original cultures were respiratory-sufficient, it is inferred that propamidine isethionate has destroyed the respiratory apparatus. The living cells in the zone around the disk have been irreversibly transformed into obligate anaerobes. This is demonstrated by inoculating the cells from the zone into a peptone yeast-extract broth containing sodium acetate as the major source of carbon. The medium contains phenol red as an indicator, and if a single cell with an intact respiratory apparatus is introduced into this medium it will grow and the medium will turn deep red due to the increase in *p*H [3]. Although cells from the area surrounding the disk grow well in glucose broth, transfers to the acetate broth never produce an alkaline reaction, proving that all the cells have lost their ability to respire acetate and hence are respiratory-deficient. The conclusion that propamidine isethionate has destroyed the respiratory apparatus irreversibly seems irrefutable. It also appears to have achieved this end with relatively little harm to the cell otherwise.

This experiment is simplified by using a yeast which is incapable of synthesizing adenine, since strains of this kind with an intact respiratory apparatus are pink while the respiratory-deficient strains are white[4]. When this experiment is performed with a pink yeast, the yeast which appears in the zone surrounding the disk is white, although it remains adenine-dependent. White cells from such a plate always fail to grow after transfer to the acetate broth, justifying the inference that all the cells have lost their respiratory apparatuses since a single cell with an intact respiratory apparatus would be able to utilize the acetate and grow in the acetate broth. A poison of this type which acts abruptly and completely on the respiratory apparatus might be assumed on Warburg's theory not to be carcinogenetic because it acts so quickly and completely that the mammalian cells (which do not have a fully formed anaerobic pathway available and waiting) would not be able to adapt themselves to anaerobic growth. The phenomenon demonstrates the extreme sensitivity of the respiratory apparatus to a poison which has little or no effect on the survival of the cell.

Warburg's theory assumes that low oxygen tension can lead to crippling of the autonomous grana and thus to cancer. Warburg's inference that respiratory deficiency could be induced by anaerobiosis was confirmed by Hino and Lindegren[5] using yeast. (Respiratory-deficient yeasts, induced by anaerobiosis or by a variety of other treatments, are completely capable of maintaining their structural integrity permanently from generation to generation, although all the cell's energy is supplied only by fermentation. Warburg, quoting Pasteur, stated that "not even yeast, which is one of the lowest forms of life, can maintain its structure permanently by fermentation alone; it degenerates to bizarre forms". This view concerning the stability of yeast grown exclusively anaerobically is in error, but this fact has no bearing on the validity of Warburg's theory of the origin of cancer, since it neither supports nor invalidates any of the assumptions upon which the theory is based.) Both Sarachek[6] and Harris[7] failed to confirm the induction of respiratory deficiency in yeast by anaerobiosis. In three haploid cultures Hino and Lindegren found that the frequencies of respiratory-deficiency under aerobiosis were, respectively, 1·7, 2·8 and 3·2 per cent, while the same cultures grown anaerobically produced, respectively, 14·9, 8·6 and 8·0 per cent. Two diploid cultures under aerobic conditions produced, respectively, 0·8 and 0·0 per cent respiratory-deficiency, and 1·9 and 0·2 per cent under anaerobic conditions. In one tetraploid culture the frequency of respiratory-deficiency rose from 0·0 under aerobiosis to 0·3 per cent under anaerobiosis. It is clear that the haploid state provides the clearest demonstration of the effects of anaerobiosis on respiratory-deficiency, and it is not surprising that neither Sarachek nor Harris detected this effect, since they did not use haploid cultures. Anaerobiosis (unlike propamidine isethionate) induces only a low frequency of respiratory-deficiency; the effect of propamidine isethionate is total. Anaerobiosis produces relatively minor damages which might be progressive, if repeated, since only a few of the grana are affected in most cells and only rarely are all destroyed. Anaerobiosis is inferred to be an effective carcinogen, since it only reduces partially the energy available from aerobiosis and thus enables the cell to survive long enough to adapt the dormant fermentative system.

Recent work in this laboratory[8] has provided an example of another kind of induction of respiratory-deficiency in yeast. Caffeine induces respiratory-deficiency in yeast cells sensitive to caffeine with much higher frequency than in cells resistant to caffeine. Growth in caffeine is only achieved by an adaptive process, presumably an enzymic adaptation. Both resistant and sensitive yeast cells pass through the adaptation; but the adaptation of sensitive cells is characterized by the simultaneous induction of an extremely large number of respiratory-deficient cells. Although one may assume that the total induction of respiratory-deficiency by propamidine isethionate is due to the direct action on the grana, the induction of respiratory-deficiency which occurs when caffeine-sensitive cells become adapted to caffeine may be due to injury of grana, not by direct action of caffeine upon the grana, but through the metabolic stress imposed by oxidative adaptation to caffeine.

The metabolic stress of adaptation can be observed cytologically. Several years ago when interest in the laboratory centred on adaptation of yeast to galactose, yeast cells were critically observed during adaptation. In the early stage of adaptation most cells were not budded and did not stain with methylene blue. (Non-stainability is the standard criterion for viability.) Just before adaptation was achieved (as indicated (1) by the absence of budding; (2) by the failure to accumulate glycogen; (3) by slow utilization of galactose) nearly all cells became stainable with methylene blue. In other circumstances this would indicate that the cells were non-viable. Shortly thereafter, however, at least 80 per cent of the cells produced buds, lost their stainability with methylene blue, and vigorous fermentation of galactose occurred. This is interpreted to mean that the stress of adaptation had exhausted most of the reducing reserves of the yeast cell, permitting it to stain with methylene blue, but that this deficiency was made up as soon as sufficient galactose became available.

An important use of Warburg's theory is for predicting the possibility that an agent is a carcinogen from its action on the respiratory grana. On this theory, propamidine isethionate is predicted not to be carcinogenetic whereas anaerobiosis and caffeine are. The dye janus green, which acts specifically to stain the respiratory grana and which is singularly effective in preventing sporulation in yeasts, is predicted to be non-carcinogenetic. Incidentally, the sporulative process in *Saccharomyces* is an especially useful point for testing the effectiveness of various toxic agents on the respiratory grana, since it is a highly specific kind of differentiation which is achieved only aerobically. The view that viruses may cause cancer can also be deduced directly from Warburg's theory. Warburg inferred that a great variety of metabolic disturbances could inactivate the respiratory apparatus, and experimental evidence from yeasts supports his views. Since many viruses grow in the cytoplasm without killing the cell, they are disturbers *par excellence* of cytoplasmic metabolism and might be expected readily to cause the destruction, inactivation or malfunction of the grana. Viruses inhibiting the nucleus would be predicted to be not nearly so effective.

Ephrussi[9] was the first to speculate concerning the chemical mechanism by which the respiratory grana of the yeast cell could be destroyed. He assumed that since acriflavine has an affinity for nucleic acids it was mutagenic and he found that it induced respiratory deficiency. Ephrussi was unaware of the fact that Stier and Castor[10] had previously induced respiratory deficiency with cyanide, and Whelton and Phaff[11] had shown that ethylene oxide is extremely efficient in producing the same effect. It has become increasingly evident that a great variety of substances induce respiratory deficiency in yeasts: copper and manganous salts are recent additions. Whether these substances act directly as propamidine isethionate appears to, or indirectly as temperature[12] and caffeine adaptation, it seems clear that study of the phenomenon should be revealing in terms of the nature of the respiratory grana and, therefore, of carcinogenesis.

This work has been supported in part by a research grant from the American Cancer Society.

[1] Warburg, O., *Science*, **123**, 309 (1956).
[2] Weinhouse, S., *Science*, **124**, 267 (1956).
[3] Tavlitzki, J., *Rev. Can. Biol.*, **10**, 48 (1951).
[4] Ogur, M., Lindegren, G., and Lindegren, C. C., *J. Bacteriol.*, **68**, 391 (1954).
[5] Hino, S., and Lindegren, C. C., *Exp. Cell Res.*, **15**, 628 (1958).
[6] Sarachek, A., *Cytologia*, **23**, 143 (1958).
[7] Harris, M., *J. Cell. and Comp. Physiol.*, **48**, 95 (1956).
[8] Maddox, P., and Lindegren, G., *Bacteriological Proc.*, abstract. 28 (1959).
[9] Ephrussi, B., *II Annales de l'Institut Pasteur*, **76**, 1 (1949).
[10] Stier, T. J. B., and Castor, J. G. B., *J. Gen. Physiol.*, **25**, 229 (1941).
[11] Whelton, R., and Phaff, H. J., *Science*, **105**, 44 (1947).
[12] Sherman, F., thesis, Univ. of Calif., Berkeley (1958).

# THE NEW MARINE BIOLOGICAL STATION ON HELIGOLAND

By Dr. J. N. CARRUTHERS
National Institute of Oceanography, Wormley, Surrey

MANY years before the island of Heligoland passed from British to German ownership in 1890, it was popular with distinguished scientists from Germany and other Continental countries for marine biological pursuits, because the waters bordering the German mainland yield only a small part of the faunal range characteristic of the open sea.

In the Baltic, the low salinity of the German waters there is a limiting factor and in the North Sea the turbidity is such. Moreover, in the North Sea the wide belt of mainland shore which dries out at low tide militates against the establishment of a first-rate German marine biological station there. Well before 1850, men whose names were to become world-famous in the domain of marine biology were frequenting Heligoland because of the much better natural conditions which exist there.

So long ago as 1835, Ehrenberg had investigated the origin of bioluminescence while working on the island and, ten years later, Johannes Müller had there formulated new ways of investigating the life of the open sea. In 1865 Anton Dohrn (later of Naples fame) and Ernst Haeckel of Jena were conducting researches from the island, and, within two years of its cession to Germany, a marine biological station had been founded with F. Heincke as first director. During the twenty-eight years of his tenure of the post, his famous work on the natural history of the herring and on the plaice was carried out. In those days the Station was housed in more than thirteen separate buildings and its own staff of eight scientists and sixteen technical assistants had to find room for sixteen guest workers. Not until it was possible (in 1920) to take possession of a large building freed from naval use did it become possible to conduct tuition courses, and the first of these was attended by a class of thirty.

Many British oceanographers will well remember Heincke's successor—the genial and talented W. Mielck, who was head of the Station during

Fig. 1. General view from the water front

1920–33. When its enlargement was completed in 1937, the name "Wilhelm-Mielck-Haus" was given to part of the multi-storied construction. The last director of the Station to be appointed before the Second World War was A. Hagmeier, who held the post on the island from 1934 until after hostilities had broken out. He remained the nominal director of the Station until his death in 1953. Until shortly before that time, he had been resident in Sylt at the dependent Ellenbogen Station near List. While there Hagmeier spent much effort in the furtherance of marine biological research and was able to adapt a vacated army building in List to provide a two-roomed laboratory on the harbour side in which instructional courses could be given. Already by 1949 the number of visiting students had reached 170. When, in 1952, the island of Heligoland was made free of access again and declared ready for building operations to start, Hagmeier was already prepared with plans for reconstituting the renowned Biologische Anstalt on it. Though it is not by any means the case that all German marine scientists were in favour of the project, the marine biologists for the most part were so.

In pre-1939 days, the "Biologische Anstalt Helgoland" came under Prussia and one may read, in old reports of the Government, of that State having furnished 400,000 marks for the rebuilding which began in the winter 1925–26. when serious thought was given to rebuilding the completely demolished Station some years after the end of the Second World War, however, the island had come under Schleswig-Holstein. This *Land* not being financially able to shoulder the expense of such an ambitious project, it came about that the *Bund* Ministry of Land, Food and Forests eventually did so.

Much space would be needed to give an adequate account of the impressive proceedings of June 19 last, when the fine new Station which has arisen on the ruins of the old was formally declared open in the presence of a large concourse headed by Bundesminister Dr. Lübcke, who has since been elected President of Western Germany in succession to Dr. Heuss. Ministers of Schleswig-Holstein were present; German oceanographers were there in number; guests from the Netherlands, Italy and Britain attended, and an English fisheries research vessel (*Sir Lancelot*) had come from Lowestoft. The Director of Fisheries Research in Britain was present, greatly to the gratification of Dr. A. Bück-

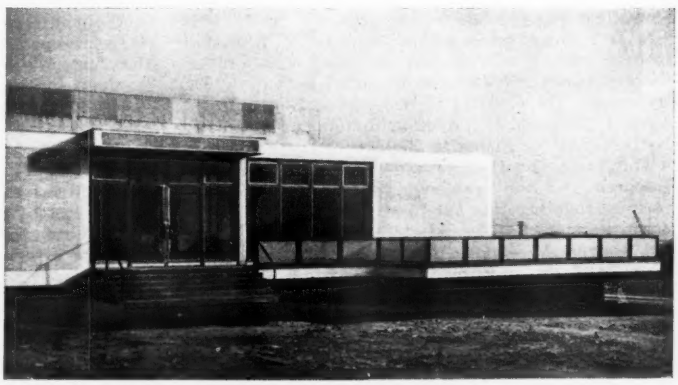


Fig. 2. Entrance to the aquarium with (on the right) the verandah around the seal basin

mann, director of the new Station, who recalled the close associations between Lowestoft and Heligoland during the years between the wars. Most of the guests had been carried from Cuxhaven in the very fine German research vessel *Anton Dohrn*, which has already much first-class work to her credit despite her youth.

Speeches and a tour of the new Station were the business of the first day—followed by a reception and supper aboard the *Anton Dohrn*. The second day (June 20) was devoted to a scientific conference in the commodious lecture room. After a *Festvortrag* by Dr. J. Verwey of Den Helder, he opened a general discussion on what should be the scientific activities of marine stations. After this topic had been debated very widely with no lack of participants, Dr. A. Bückmann gave a very detailed account of the programme of work which he has in view for his fine new Station. From what he said it was quite clear that great care had been taken to build as economically as possible in erecting the long low buildings which are such a contrast to those standing when the former building programme ceased in 1937. Because practically everything had to be transported from the mainland at great additional expense and with dependence upon weather, it is perhaps not surprising that the cost to the *Bund* Ministry was in the neighbourhood of six million D.M. (about £6/11 million).

The divisions of activity provided for under the general leadership of Dr. A. Bückmann, who remains resident in Hamburg, where he is professor of fisheries biology in the University of Hamburg, are : zoology, animal physiology, botany, microbiology, planktonology and fisheries biology. The first of these divisions has a staff of three scientists with the Director as leader. The other divisions have two scientists each, except for microbiology, which has only one. It seemed surprising that there is no special provision for the hydrographical work which is essential before a proper study can be made of the relationships between the marine fauna and flora and the environment. An island like Heligoland must afford wonderful opportunities for studying what differences exist in the marine life as between what must usually be the lee and weather sides.

There are many things of great interest with which a worker trained in the physical side of oceanography could occupy himself working from the new Station. Questions elicited the remarks that expense had to stop somewhere, but that visiting workers on any aspect of oceanography would always be made most welcome—as evidenced by the great amount of laboratory space set aside for guests.

It is a very praiseworthy feature of post-War German marine science that excellent collaboration exists between the Heligoland activities led by Dr. Bückmann, the German Hydrographic Institute, the German Scientific Commission for Marine Research, the Institute for Fisheries Biology of the University of Hamburg, the Bremerhaven Institute for Marine Research and the University of Kiel. This close working together has led to really notable achievements in respect of investigations on the high seas, and German oceanographical investigations made during the Polar Front Survey within the International Geophysical Year programme are of high merit.

Along the southern face of the main block of the new Station are the laboratories in use by the staff and the rooms used for the dispatch of animals to universities and other customers. The northern face contains the laboratories for guest workers and students. The public aquarium, with all its complicated technical 'plumbing', and the aquaria for research and growth studies constitute a separate block. An impressive feature of the aquarium which those with memories of the pre-War Station will recall has been provided anew and in duplicate. Quite detached and centrally placed are a pair of circular 'tanks' in the form of very large glass vertical cylinders rising to a considerable height from the floor. Co-axially placed within each of them is an opaque vertical cylinder of considerable diameter which has a sanded surface. As a result, fast-swimming fish such as herring, mackerel and hake can swim swiftly around and around in the annular water space without knocking into the external glass or being crowded into corners. It is a wonderful sight to see the fish incessantly swimming around on their (to them) endless journey. Great value is attached to the fine supply of excellent sea-water pumped in from the end of a mole, and to the fact that all the 'plumbing' is of plastics. One sees handsome arrays of bright green and red piping more or less everywhere. The Station's cutter *Uthorn*, which is 24·5 metres long, of 6·4 metres beam, and of draught 2·6 metres, does 8 knots under the drive of her 150 h.p. engine. She is excellently equipped with instruments and is of great use in enabling the scientists to dredge in the Heligoland Rinne, which is one of the deepest parts of the southern North Sea and has a fauna of particular interest. Two motor-boats are also available.

It was emphasized that to run a marine biological station to-day is a vastly different affair from what it was in Anton Dohrn's day. The mass of expensive and costly apparatus which had already become necessary a decade and more back has been much added to by the requirements for work with carbon-14 and other isotopes. The new Heligoland Station is well equipped for work with eight or more tracer elements.

Heligoland was always a favourite holiday resort, and already some eleven hundred permanent inhabitants are housed again on the island to make the most of it. 'Day tripping' from the mainland is also a very intense and paying business. During the summer the multitude of visitors contribute to the Station's funds by way of their payments to visit the aquarium. In winter when the visitors no longer come, the scientists of the Station can use the large annular 'tanks' described above of 25 m.$^3$ capacity for studies on the swimming of migratory fish.

About fifteen guest investigators pursuing quite different lines of work can work in association with the thirteen scientists of the Station, and great help is given in the matter of lodging to guests whose visits take place in summer. By throwing two large classrooms into one, thanks to a movable partition, a lecture room with ample accommodation for fifty students becomes available. Provision is made for visiting university teachers to bring students to the Heligoland Station for classes lasting some weeks, and facilities are afforded for teachers of biology and others to attend refresher couses.

The complex of buildings, which has a total frontage of 120 metres, includes a special basin in which a seal will live. This, with glass 4 cm. thick, is most ingeniously constructed. Outside, walking on a balcony, paying visitors will be able to look down on the seal from above. From inside the building the visitors will look into the tank illuminated by daylight from

above, and will see many smart movements imposed upon the seal by the clever internal 'architecture' of the tank. This fine basin recalled the modest accommodation which the seal living in the Heligoland aquarium many years ago had to be content with.

As with the Station of former times, the very appropriate speech from Goethe is in evidence. It now faces us set into the wall in large bronze capitals as we enter through the main public door: "Alles ist aus dem Wasser Entsprungen, alles wird durch das Wasser erhalten, Ozean gönn uns Dein ewiges Walten".

It was due to the kind generosity of the *Bund* Ministry that I was able to attend the impressive re-opening. For those who would know very much more about the new Biologische Anstalt Helgoland, a full account written by its director exists[1] and a shorter one by one of his assistants[2].

[1] Bückmann, A., *Helgol. Wiss. Meeresunters.*, **7**, Heft 1, 1–50 (Hamburg, 1958).
[2] Hempel, G., *Die Umschau*, **12**, 353–4 (Frankfurt am Main, 1959).

# OBITUARIES

## Prof. E. Percival

A GREAT blow has been dealt to marine and freshwater biology in New Zealand by the death on July 15, in Christchurch, of Prof. Edward Percival. Prof. Percival, who was born in 1893, was elected to the chair of biology at Canterbury University College, as it was then called, in 1928 after serving as lecturer in the Department of Zoology at the University of Leeds, where he was assistant to the late Prof. Walter Garstang. During 1928 he worked as a temporary naturalist in the Plymouth Laboratory on the ecology of the Rivers Tamar and Lynher, and he will be well remembered by those who knew him then. The son of a Cheshire farmer, Percival spent his boyhood in Lancashire, where he took the national diploma in agriculture at the Harris Institute, Preston, and he found much to interest him when he came to New Zealand. His early work on the ecology of rivers in Yorkshire led him to play a prominent part in trout management in acclimatization society work in New Zealand, and his experience in marine biology in the United Kingdom was put to good use in guiding his research students and in advising the policies of various government departments.

His published work, amounting only to about a score of papers, is not a sufficient criterion by which to judge the man. He had declined various academic honours since he maintained that such things were of no use to him. He did, however, consent to be elected a Fellow of the Royal Society of New Zealand.

First and foremost he was a teacher. From the elementary- to the postgraduate-level he never failed to bring out the best in the human material set before him. His particular philosophies on the teaching and appreciation of biology will be long remembered by all those who came in contact with him, even if only as incidental associates.

For his advanced students he held a twice-yearly field expedition to Menzies Bay, one of the isolated little coves on Banks Peninsula, and he took his students into the field on almost every other weekend during term, bringing a certain spartan approach to the pleasures of animal observation which helped one more fully realize what is meant by ecology. His aim was to produce, at the postgraduate-level, a student well balanced in outlook, able to think, not to be a storehouse of facts but to know where to turn to find what is already known, and potentially able to go on in almost any field of biological endeavour. How successful he has been in this can be seen by the wide distribution of his honours students in various positions throughout the world.

The informal, and often quite unzoological, discussions which he held in the field and in the well-remembered atmosphere of his rooms, together with his novel methods of allowing notes to be taken into the examination room, all helped to bring out what powers of expression and thought were available in his students. Indeed, he often remarked that he wished he could conduct his examinations in the University library, for he would know even more easily the worth of his candidates.

Physically he was outstanding for a man near retirement, and in the field his stride and energy in every activity proved more than a match for even the most athletic of his followers.

At the first encounter he presented a rather forbidding aspect and was inclined to be of uncertain temperament, but this, particularly in his later years, was a variation on the theme of not suffering fools gladly. If one genuinely wanted help, advice or encouragement and if one had exercised all one's resources before approaching him, Percival became the proverbial tower of strength and it was difficult not to find oneself being cast in his mould.

Apart from his long-term studies on lakes in the Canterbury foothills and his interests in marine matters, his scientific work will long be remembered for his fine studies on the embryology of the Brachiopoda. Percival was fortunate in having a locality close by where these animals could easily be taken between tides on the rocky shore and he was able to use his advantage to the full in producing his studies on their development and growth. He was never afraid to admit that he was wrong or that he did not know, an attitude of mind which had its effects particularly on his elementary students contemplating a career in teaching.

Percival's influence extended far beyond the cloisters of the University of Canterbury, and it would be hard to exaggerate just how widely his teaching and stimulation have been, and will continue to be, felt. ELLIOT W. DAWSON

## Dr. M. R. Schafroth

DR. MAX ROBERT SCHAFROTH and his wife, Käthi Schafroth (*née* Gemperle), died on May 29; they were killed in an aeroplane crash in Northern Queensland, Australia.

Dr. Schafroth was born in Burgdorf, Switzerland, on February 8, 1923. He passed his matriculation at the Gymnasium in Bern in 1949. He then entered the University of Bern in order to study mathematics and physics, later changing to the

Swiss Federal Institute of Technology in Zurich. He graduated at the latter in 1948, obtaining a diploma in mathematics and physics.

Thereafter he commenced research work under the direction of Prof. W. Pauli, who was professor of theoretical physics at the Institute and Nobel prize-winner of 1944. Dr. Schafroth obtained his doctorate degree in 1949 and was thereafter appointed as assistant to Prof. Pauli—a post he held until 1953. During that time he continued research in collaboration with Prof. Pauli in quantum field theory and solid state physics.

At this stage the Schweizer Arbeitsgemeinschaft für Mathematik und Physik offered him an overseas travel grant for two years, which he decided to spend in the Department of Theoretical Physics of the University of Liverpool with Prof. H. Fröhlich. After one year in Liverpool, however, he left Europe to accept a lectureship offered him by Prof. H. Messel in the then newly expanded School of Physics at the University of Sydney. He remained at the University of Sydney until his death, having been promoted to a senior lectureship on January 1, 1955, and to a readership on January 1, 1957. In May 1958 he was invited to accept the chair of theoretical physics at the University of Geneva, Switzerland, which invitation he had accepted and intended taking up this post on September 1, 1959.

Starting mainly with his stay with Prof. Fröhlich in Liverpool, Dr. Schafroth became interested in the theoretical understanding of the phenomenon of superconductivity. This was also his chief research interest during his five years at the University of Sydney. He published several penetrating papers on this subject himself and also inspired contributions from others in the School. There can be no doubt that his work in this field will go down in science as having been a major contribution to the understanding of superconductivity. Several papers by him are yet to appear. These include a review of the field in the series "Solid State Physics", edited by Profs. Seitz and Turnbull.

Apart from this particular field of research, Dr. Schafroth was an expert and inspiring lecturer in the fields of statistical mechanics, solid state physics in general and quantum field theory. He brought into his lectures something of the qualities of his old teacher, Prof. Pauli.

The death of Robert Schafroth and of his wife Käthi will be a great loss to all who knew them.

S. T. Butler

## Mr. H. W. Greenwood

The death of Mr. H. W. Greenwood occurred on April 30 at the age of seventy-seven. In the course of a very active life, Mr. Greenwood was associated with three industries. Before the First World War he was a mining plant superintendent in the south of Spain. In 1919 he joined Leto Photo Materials Company, manufacturers of photographic paper, and continued with the firm after their amalgamation with Wellington Ward and their later amalgamation with Ilford, Ltd. Since 1937 he had been associated with Powder Metallurgy, Ltd.

Mr. Greenwood wrote profusely in all three subjects, papers and books of a popular but well-informed nature. For many years he was an important contributor to the *British Journal of Photography*.

W. D. Jones

# NEWS and VIEWS

## Electrical Engineering at Newcastle upon Tyne : Prof. J. C. Prescott

Prof. J. C. Prescott, professor of electrical engineering at King's College, Newcastle upon Tyne, retires this year. He was elected in 1937, succeeding W. M. Thornton, who was the first holder of the chair. After studying at the University of Liverpool under Prof. E. W. Marchant, he entered a college apprenticeship with the British Westinghouse Company in 1915, continuing later with that Company as research engineer. He saw service with the R.N.V.R. during the First World War, being attached to H.M. Mining School at Portsmouth. After the War, he returned to the University of Liverpool as lecturer in electrical engineering, where he was to remain for 18 years. His early researches were concerned with the behaviour of constant-current dynamos, and this led by way of a study of the free period of coupled alternators to researches on the inherent instability of parallel connected synchronous electrical machinery. His papers of this period reveal that electrical measurement and measuring instruments were also occupying a substantial part of his time. For this work he was awarded the degree of doctor of engineering by the University in 1931. In Newcastle his research work has been concerned with synchronous governing of alternators, and further studies of the stability of parallel-connected alternators have been made; latterly he has been engaged in an investigation into the performance of turbo/alternator governors, which is still in progress.

The Department of Electrical Engineering at Newcastle has grown greatly in size during Prof. Prescott's tenure of the chair. Under his direction the expansion has been conducted so as to preserve a balance between so-called 'light' and 'heavy current' electrical engineering, and to avoid too early specialization in undergraduate courses. Always playing a large part in the teaching activities of his Department, he has consistently emphasized the necessity for the teaching of fundamentals in university courses, and has always insisted upon the maintenance of a high academic standard in his Honours School. Like his predecessor, he has been active in the affairs of the Institution of Electrical Engineers, being chairman of the North Eastern Centre in 1943–44, and has taken a continued interest in the North East Radio and Measurements Group. Coming from a literary family, he is a man of uncommonly wide interests who holds the respect and affection of all who know him. His friends both inside and outside the University wish him many years of active and happy retirement.

## Dr. R. L. Russell

Dr. R. L. Russell, who has been appointed to the chair of electrical engineering at King's College, Newcastle upon Tyne, in succession to Prof. J. C. Prescott, graduated B.Sc. in 1938 and M.Sc. in 1939,

in mathematics at the University of Leeds. Soon afterwards, he took up work in the Admiralty Degaussing Department at Portsmouth and Helensburgh, and thus his first contacts with electrical engineering were made. In 1942, when the magnetic mine had been overcome and there was an acute demand for radio mechanics, Dr. Russell accepted lectureships on radio training courses, first at the Royal College of Science and Technology, Glasgow, and then at Robert Gordon's Technical College, Aberdeen. He went from Aberdeen to the Research Department of the British Thomson-Houston Co. at Rugby. In 1946, he was appointed as lecturer in the Department of Electrical Engineering in the University of Bristol, and in 1955 he was promoted to a readership.

Towards the end of the War he published certain papers of a semi-geometrical nature, mainly arising out of problems in radio engineering. As time has proceeded, he has turned his essentially geometrical mind to a number of problems, first in electrical measurements and then in relation to electrical machines. Many papers have been published in the *Proceedings of the Institution of Electrical Engineers* arising out of this work, which led to Russell receiving the degree of D.Sc. from the University of Leeds a few months ago. Dr. Russell's most individual contribution to electrical engineering has been to observe the possibilities which arise from feeding a three-phase winding simultaneously at both ends with voltages of different frequencies. This principle has already had several applications, and it is of interest to note that Russell's predecessor in Newcastle, Prof. J. C. Prescott, adopted the idea in relation to synchronous governing. A recent Ph.D. thesis on synchronous governing presented at King's College incorporated just such a double-fed device.

## Agricultural Botany at Leeds: Prof. J. H. Western

Dr. J. H. Western has been appointed to the newly instituted chair of agricultural botany within the Department of Agriculture in the University of Leeds. He was educated at Avoncroft Agricultural College, Evesham, and the University College of Wales, Aberystwyth, where he graduated in botany with agricultural botany. In 1937 he was awarded the degree of Ph.D., the subject of his thesis being "Some Aspects of Biological Specialization in the Oat Smut Fungi". In that year, he also took up an appointment at the Welsh Plant Breeding Station, Aberystwyth, where he undertook research on diseases affecting herbage plants. In 1939 he was appointed lecturer in agricultural botany and adviser in mycology in the University of Manchester, leaving in 1946 to take up appointment as provincial plant pathologist to the Northern Province, Ministry of Agriculture and Fisheries, Newcastle upon Tyne. In 1951 he was appointed to his present post of senior lecturer in agricultural botany in the Department of Agriculture in the University of Leeds. His investigations have included the problem of the 'choke' disease of cocksfoot (*Dactylis glomerata*) caused by the fungus *Epichloe typhina*, and he has been responsible for numerous publications.

## Physiology at King's College, London: Prof. R. J. S. McDowall

The retirement is announced of Prof. R. J. S. McDowall from the Halliburton chair of physiology at King's College, London. This he has held for thirty-six years. He has perhaps become best known for his books. The most outstanding is "Control of the Circulation of the Blood", a monumental work with more than 9,000 references; but his "Handbook of Physiology", of which he has produced eleven editions, has been the bible of a generation of medical students. His "Sane Psychology" has been reprinted four times. Prof. McDowall's interests have been chiefly in the circulatory system in which he has been an untiring worker and is a recognized authority. He gave the Oliver Sharpey Lecture of the Royal College of Physicians in 1939 on this subject. He has also been largely responsible for the formation of the Asthma Research Council and in recognition of this he was made president of the fourth European Congress of Allergy held in London in September. His enthusiasm and powers of inspiration are reflected by the fact that thirteen of his pupils have become professors and, of these, six have been in the University of London.

## Prof. J. L. D'Silva

Prof. J. L. D'Silva has been appointed to succeed Prof. R. J. S. McDowall. Prof. D'Silva first graduated in 1929 from King's College in chemistry. His early interests were in organic chemistry and he was elected Sir Halley Stewart Fellow in 1933. His attentions then turned to physiology, and appointments leading to the readership in physiology at St. Bartholomew's Hospital Medical College followed. Here he pursued his particular interests in the effects of adrenaline and adrenaline-like substances on serum electrolytes. In 1948 he was appointed to the chair of physiology at the London Hospital Medical College where his research contributions to the understanding of respiratory mechanics again reflected his early interest in the physical sciences.

## University of Malaya in Kuala Lumpur: Prof. R. S. Huang

In the article under the title "University of Malaya in Kuala Lumpur" in *Nature* of August 1, p. 306, it was stated that Prof. R. A. Robinson had been appointed to the chair of chemistry. It has now been announced that Prof. Robinson has declined the appointment, which has been accepted by Prof. R. L. Huang, reader in chemistry in the University.

Prof. Rayson Lisung Huang was educated in Hong Kong, first at Munsang College and then at the University of Hong Kong. After a year as demonstrator in chemistry at Kwangsi University, China, Dr. Huang went to the University of Oxford with two scholarships, from the Rhodes Trust and the British Council, to study under Sir Robert Robertson. He gained his doctorate as a result of this period of research on synthetic hormones. Dr. Huang then visited the University of Chicago with a postdoctoral fellowship and studied for two years under the late Prof. M. S. Karasch, and then joined Prof. Konrad Bloch as a research associate for a further year. During these periods Dr. Huang worked on free radicals and on the biosynthesis of cholesterol, respectively. He joined the University of Malaya early in 1951 and has been awarded the degree of D.Sc. for his researches mainly in the field of free radicals. A citizen of the United Kingdom and Colonies, Dr. Huang has a good knowledge of classical and modern Chinese, and speaks several Chinese dialects. His scholarship in a wide field will be a most valuable contribution to the professoriate of the new Division of the University of Malaya.

## The Second Russian Space Rocket

A Russian multi-stage rocket was launched at about 9 hr. U.T. on September 12 and its final stage, weighing 1,511 kgm. (3,331 lb.) when empty of fuel, was projected towards the Moon with a speed of 7 miles per sec. At 18h. 40m. U.T. on September 12, when it was about 90,000 miles from the Earth, the vehicle emitted a cloud of sodium vapour. This was observed from eastern Europe and Asia as a glow, which lasted about 5 min. and was of stellar magnitude 5, in the constellation of Aquarius. At an unspecified point on its journey, the vehicle divided into two parts, an instrumented sphere weighing 390·2 kgm. (860 lb.) and the spent rocket. The sphere carried instruments to measure the magnetic fields of the Earth and Moon, the zones of radiation around the Earth, cosmic rays in space, the impacts of micrometeorites and the composition of interplanetary gas. It was stated that these experiments were successfully accomplished. The vehicle carried radio transmitters operating on some of, or all, the frequencies 19·993, 19·997, 20·003, 39·986 and 183·6 Mc./s. The radio signals ceased abruptly at 21h. 02m. 24s. U.T. on September 13, when the instrumented sphere struck the surface of the Moon at long. 0°, lat. 30° N., near the crater Archimedes.

While the vehicle was above the horizon in Britain, it was tracked by the Jodrell Bank radio telescope: the measurements made, of the direction, the Doppler frequency and the moment of impact, all confirmed that the vehicle followed very closely the trajectory given by the Russians. This second space rocket was similar to the first (launched on January 2, 1959; see *Nature*, **183**, 83), which had a total weight of 3,245 lb., including 797 lb. of instruments in a spherical container, and performed similar experiments. The trajectories of the two rockets were also similar—both took about 35 hr. to reach the vicinity of the Moon—though the first rocket, instead of colliding with the Moon, flew past it to become the first artificial planet.

## Rocket Studies of Emissions caused by Solar Flares: National Science Foundation Grant

The National Science Foundation has made a grant of 250,000 dollars to the U.S. Naval Research Laboratory for rocket observations of ultra-violet and X-ray emissions from solar flares. Principal investigator of the project is Dr. Herbert Friedman, superintendent of the Atmosphere and Astrophysics Division of the National Research Laboratory. The effect of the Sun's emissions upon the ionosphere and the resulting radio communication problems are of great theoretical and practical significance. Measurement and understanding of the origin of these emissions will be basic to an understanding of the solar flares themselves. Dr. Friedman will therefore launch a series of instrumented *Nike-Asp* rockets to make the necessary observations. The investigation will explore wave-lengths and altitudes at which only rudimentary observations have so far been made. Previous work by Dr. Friedman, Dr. Richard Tousey, and their co-workers at the National Research Laboratory has established the existence of intense radiations from the Sun in the wave-length regions under consideration. Dr. Friedman and his group at the Naval Research Laboratory were pioneers in the use of rockets for astronomical research, and astronomers credit his work with being among the most exciting and significant at present being carried on in the field. Instrumentation for the series of rocket observations will include Geiger counters to measure X-rays in the 1–10 A. range, X-ray detectors for wave-lengths of 8–20 and 44–60 A., scintillation counters sensitive to hard X-rays in the 20–500 kV. range, and ion chambers sensitive to the helium emission lines at 584 and 314 A.

## British Aid for Nato Scientific Courses

Mr. H. Nicholls, Parliamentary Secretary to the Ministry of Works, stated in the House of Commons that the United Kingdom contribution to the fund established by the North Atlantic Council to promote international gatherings for the advanced study of special scientific topics would be about £7,000 in the first year and would be borne on the vote of the Department of Scientific and Industrial Research. This was a written answer on June 25 in response to a request for a statement regarding United Kingdom participation in the new North Atlantic Treaty Organization scheme for advanced study institutes. The funds would mostly be used for contributions to the teaching and administrative expenses of selected courses at institutes in a Nato country which offered intensive courses, usually at postdoctoral to professional level, in branches of the natural sciences and technology. Assistance might also be given to the travelling and scholastic expenses of participants from Nato countries.

## Graduates and National Service

In answering a question in the House of Commons on June 8, the Minister of Labour, Mr. Ian MacLeod, said that he was not prepared to extend the deferment arrangements for science and engineering graduates to men with other qualifications. This was the advice of his Technical Personnel Committee, which had considered last autumn difficulties said to be created for firms engaged in nuclear engineering and other industries by the call-up of non-graduates engaged on research. It was extremely difficult to distinguish between all the different professional qualifications that non-graduates have, but Mr. MacLeod said he would be willing to receive a deputation of research directors to discuss a definition which might be as effective as the graduate definition, if Mr. Blenkinsop, his questioner, cared to organize such a deputation.

## Research and Development Charges in Civil Nuclear Power

A question was asked in the House of Commons on June 15 regarding the proportion of research and development charges for the civil nuclear power programme borne by public funds through the Atomic Energy Authority. The Paymaster General, Mr. H. Maudling, in reply said that all research and development expenditure incurred by the Atomic Energy Authority was originally borne from public funds. However, it was intended that the expenditure attributable to the civil power programme should be recovered in due course in the form of royalties payable on the electricity stations and as part of the price charged for fuel elements. The Authority also recovered the cost of the consulting services from the electricity boards.

## Nuclear Reactor *RB* Accident in Yugoslavia

Volume 9 of the Bulletin of the Boris Kidrich Institute of Nuclear Sciences contains twenty-five articles and two laboratory notes of technical interest contributed by members of the physics, physical

chemistry and radiobiology laboratories of the Institute. They are prefaced by a statement in French by the director of the Institute, Prof. P. Savić, concerning the unfortunate accident which occurred to the nuclear reactor, *RB*, on October 15, 1958, at the Institute. Six people, who were very close to the reactor, received strong doses of neutron and ionizing radiations and two others, who were not so close, doses above the permissible level. The six were given medical treatment at the Curie Hospital in Paris, but one, V. Zivota, a nuclear physicist, died on November 15. The reactor, *RB*, is of zero energy and is fuelled with natural uranium and moderated with heavy water. Details of the construction of the reactor are given in the first article in the volume. The safety system consists of a control key, safety rods, alarm dose-rate meters and an automatic shut down. The approach to criticality is made by gradually raising the heavy-water level. The accident occurred when the reactor went out of control in becoming critical. Prof. Savić includes in his statement the conclusions of the committee of inquiry set up by the president of the Federal Commission for Nuclear Energy to report on the accident. On October 15, the committee reports, neither the alarm dose-rate meters nor the automatic control were functioning, and the personnel were judging the state of the reactor by the amount of ozone they could smell in the air. The rise in power of the reactor had been detected by the strong increase in gamma-radiation, within an interval of ten minutes, on automatic recorders of the activity in the atmosphere, placed at 540 metres from, and in direct line with, the reactor. An estimate of the radiations received by the injured persons is given in the article and shows that a total dose of about 683 rems was received, of which about 388 rems was by neutrons. The amount received individually varied according to the distance of the person from the reactor, and was about 15 per cent less than the quoted value for the farthest distant.

## The Japanese Nuclear Power Station

THE March number of the General Electric Company's *Atomic Energy Review* (2, No. 1) includes an artist's impression of the 150-MW. nuclear power station designed to be erected at Tokai Mura, 65 miles north-east of Tokyo, and which will be Japan's first nuclear power station. The General Electric Co. Ltd. has been selected exclusively to negotiate a contract for its erection. The station will be powered by a single gas-cooled graphite-moderated reactor of the same basic type as the two reactors at present being built at Hunterston, but will include many novel features, particularly with regard to structural and control considerations. It will take approximately four years to build and is expected to be in operation by mid-1963. Other articles in the issue include a description of the General Electric Company's atomic energy division by K. J. Wootton, manager of the division; a discussion of the first sixteen months civil engineering construction at Hunterston by F. W. Evans; examples of corrosion problems in gas-cooled reactors by M. W. Davies; and an account of two methods of reducing the permeability of reactor-quality graphite by D. A. Boyland.

## Technical Books

THE Atomic Energy Commission of the United States has issued a catalogue of 86 technical books published by the Commission, 1947–59. (Technical Information Service. Technical Books sponsored by the U.S. Atomic Energy Commission. Pp. 40. Washington, D.C.: United States Atomic Energy Commission, 1959.) The list is arranged by subjects and the contents of each volume are indicated. The second part of the catalogue similarly lists 26 books in the press or in preparation on April 1, 1959.

## Nutrition Meeting

THE Nutrition Meeting for Europe of the Food and Agriculture Organization of the United Nations at Rome, June 23–28, of which the report has now been published (Report Series No. 21: Report of the Food and Agriculture Organization Nutrition Meeting in Europe, Rome, Italy, 23–28 June 1958. Pp. ix+28. Rome: Food and Agriculture Organization of the United Nations; London: H.M. Stationery Office, 1958; 2*s*. 6*d*.; 0.50 dollar), was concerned with food consumption with special reference to fat consumption and with education and training in nutrition. With regard to the first, the meeting recommended the Organization to take all possible steps to foster improvements in the techniques of food consumption surveys, and of the reporting and analysis of their results, so as to ensure their maximum utility. It also recommended periodical meetings of European nutrition workers and that an expert committee or study group, in co-operation with the World Health Organization, should consider the problem of fat consumption and coronary disease in European and other countries. The need for further extensive studies of the fatty acid content of foods is emphasized in the report and also of further research to establish satisfactory tables for fatty acid composition. The present situation was reviewed with respect to education and training in nutrition, stressing the lack of suitably trained teaching staff. The contact between competent research groups, educational authorities and teachers was also considered. Further, reference was made to the narrow and unbalanced approach in teaching nutrition and the absence of refresher courses for teachers dealing exclusively or incidentally with nutrition. The meeting strongly supported the plea for a seminar in 1959 to study these questions in greater detail and to suggest better practical approaches. The main purpose of the seminar would be to examine the scope and effectiveness of education and training in nutrition in Europe. It would also formulate proposals for developing and orientating such training by government departments and other agencies, and to promote co-operation and co-ordination between the disciplines and agencies concerned and between those engaged in training and research. The report outlines an agenda for a seminar of 12–14 days for 50 to 60 people.

## Central African Scientific Research

A FURTHER 105 papers published in 1957 by members of the staff and research workers associated with the Institute for Scientific Research in Central Africa are listed in the second part of the tenth annual report of the Institute, making a total of 792. Brief abstracts of these papers constitute the rest of this part of the report (Institut pour la Recherche Scientifique en Afrique Centrale. Dixième Rapport Annuel, 1957. Pp. 228. Bruxelles: Institut pour la Recherche Scientifique en Afrique Centrale, 1959). In the administrative report, which is illustrated, the director, Prof. L. van den Berghe, indi-

cates briefly the general character of the scientific work of the Institute during the year. Prof. van den Berghe continued his own study of the biology of the tsetse flies of Bugesera and Mimuli (Ruanda) and at Jrangi, and also on the sexual and asexual cycles of *Plasmodium atherui*. Other work was concerned with the isolation of factors of growth of trypanosomes, and a histopathological study of the hypophysis of small African mammals. In nutrition, besides an extensive study of maternal milk and the variation of its content of amino-acids and proteins as a function of the period of lactation, the nutritional value of native beers was also investigated in the region of Lake Kivu and Ruanda-Urundi and the experimental kwashiorkor of swine was studied. An investigation of the fauna of the Belgian Congo and Ruanda-Urundi was launched and also of the methods and seasons of reproduction of the birds of Tshibati. In physical anthropology a study of the growth of Africans in Ruanda-Urundi was initiated and besides an inquiry into the rural economy of Ruanda-Urundi, one was commenced into the low birth-rate of the people of the Mongo tribe. In the physical sciences observations of solar radiation and of the radio-electric activity of the Sun continued, as well as studies of the functional efficiency of dwelling houses at Bagira, Kabunambo and Usumba.

## International Council of Scientific Unions

The Yearbook of the International Council of Scientific Unions, 1959 (Pp. 77. The Hague: International Council of Scientific Unions, 1959), provides a comprehensive reference work on the unions. Besides lists of members of the Executive Board, national members of the Council, of countries adhering to the Union, and of officers of the International Scientific Unions, it gives the membership of the Special Committees and other organs of the International Council. There is also an alphabetical list of these officers and members, the Statutes and Rules of the Council, the text of the agreement between the International Council and the United Nations Educational, Scientific and Cultural Organization and the reports for 1957–58 of the Secretary-General of the International Council. The Commissions of the Unions are also detailed and there is a calendar of arrangements for 1959.

No. 2 of Volume 1 (1959) of the *International Council of Scientific Unions Review* includes D. L. V. Berkner's presidential address to the eighth General Assembly of the Council. It also includes the remarks of Sir Harold Spencer Jones, the secretary general, on some affairs of the Council and the reports of the Special Committees for the International Geophysical Year, for Oceanographic Research and for Antarctic Research. The constitution of the Committee on Contamination by Extra-Terrestrial Exploration and of the Committee on Space Research is recorded and the report of the former committee is also given. This Committee believes that there is a real danger that exploration attempts made within the next few years may produce contamination of extra-terrestrial bodies, which would complicate or render impossible more detailed studies when the technological problems of landing sensitive scientific instruments on the Moon and planets have been solved. It recommends that a specific code of conduct representing a reasonable compromise between the early initiation of lunar and planetary exploration and the need to safeguard future research should be drafted with the minimum of delay.

## Council for the Preservation of Rural England

The twenty-eighth annual report of the executive of the Sheffield and Peak District Branch of the Council for the Preservation of Rural England for the year 1959 (Pp. 28. Sheffield: Council for the Preservation of Rural England, Sheffield and Peak District Branch, 1959) is seriously concerned as to the preservation of the National Parks. It emphasizes the importance of public opinion exerting effective pressure through such bodies as the Council for the Preservation of Rural England if the Peak District National Park, and other national parks, are not to be seriously damaged. At the present time the executive is seeking to prevent the desecration of the Manifold valley by a motor road and is opposing a major attack on the Green Belt of the Sheffield Development Plan—at Middlewood in the Don valley. With the local authorities and others the Branch vigorously opposed the proposals to prospect for opencast coal in the Troway valley, but the recent Government statement that areas of natural beauty will no longer be marred for this purpose should put an end to prospecting in this area. It is pointed out, however, that absence of national funds prevented the Peak Park Branch from petitioning against the Waterworks Bill which proposes to submerge the Oler valley, one of the few remaining valleys leading to the heights of the Peak District National Park.

## Society of Environmental Engineers

A Society of Environmental Engineers has been formed to provide a forum, by meetings, publications and visits, for the exchange of information and views among those engineers who are concerned with the development of equipment to withstand shock, vibration and other forms of environmental conditions, and who carry out research in these fields. The first meeting was held in London on May 29. Some fifty members and guests attended and papers reviewing the field of work were presented by Mr. D. A. Nutt (Armstrong Whitworth Aircraft, Ltd.), Mr. F. I. L. Knowles (Ministry of Supply) and Dr. P. Grootenhuis (Imperial College of Science and Technology). Future meetings are to be on November 25 and February 17 at the Imperial College. The first annual general meeting of the Society will be held on March 30 at the Royal Society of Arts. Further information can be obtained from the Secretary, Society of Environmental Engineers, 42 Manchester Street, London, W.1.

## Progress of Chemical Engineering

In the presidential address to the Institution of Chemical Engineers on April 28, Sir Hugh Beaver discussed the development and progress of chemical engineering in Britain, particularly in comparison with other countries. Pointing out that chemical engineering was now acknowledged as the fourth great technology, Sir Hugh thought that the Zuckerman Committee's estimate of our requirements of chemical engineers was too low. The figure of about 4,400 was below the present membership of the Institution—5,900 in 1959, and recent calculations of membership put the figure for 1965 at 8,800 and for 1967 at 11,100. Although the rate of increase had changed greatly in the past eight years, only in the past two or three had it approached the rate of increase in the United States. All these calculations, however, involved a change of attitude and policy as

well as of methods in British industry as would enable industry to absorb these numbers, and Sir Hugh regarded the increased application of chemical engineering as a measure of the use of modern techniques of manufacture. Referring to the training of the chemical engineer, Sir Hugh favoured our system of three, or at most four, years college education followed by practical training. He also said that the works training must be scientific and methodical, carefully thought out and systematically applied, and the system of education, while avoiding the specialization of the Continent, must produce a sufficient proportion of research students. None of the industrialists he consulted had any doubts as to the need for a broad-based training, but Sir Hugh stressed the need for an open mind on this subject. With regard to research, he felt that much more should be sponsored, especially industry. Further, liaison between industry and university was essential for our national progress.

## Haida Carvers

A DEPOSIT of the hard, dark shale called argillite was discovered in the Queen Charlotte Islands in the 1820's, and Haida Indian carvers soon began to exploit it as a material for a variety of objects, which they sold as curios. This has continued to the present day. Many of the objects are miniature copies of the larger carvings in wood, especially totem poles and chests, but tobacco pipes, bowls and plates were often made and are common in museum collections. There are also rarer objects, such as flutes.

Prof. Marius Barbeau is well known for his work on the North-West Coast Indians, and has recently published another of his many monographs on the subject (Canada: Department of Northern Affairs and National Resources. National Museum of Canada. Bulletin No. 139: "Haida Carvers in Argillite". By Marius Barbeau. Pp. viii + 214. Ottawa: Queen's Printer, 1957. 3 dollars). It is a sequel to his previous book, "Haida Myths illustrated in Argillite Carvings", and it deals, as its title implies, chiefly with the carvers themselves, but it is a complete work in itself. It is packed with information, but is somewhat haphazardly arranged. There are numerous illustrations but no list of them, and there is a table of contents but no index; the nature of the material puts difficulties in the way of providing an adequate index, but the book would have been easier if a list of the objects illustrated had been given, grouped according to their present location. A brief account of the carvers at the village of Skidegate is followed by five sections, each dealing with a particular type of object, and the remainder consists of notes on individual carvers. The book is full of interest, and the carvers, many of whom the author has known personally, live again under his hand. There has been a tendency to deprecate these argillite carvings, on the ground that they were mostly made for sale and hence were a product of acculturation and not truly indigenous. Prof. Barbeau has done a great service in directing attention to their value as works of art in their own right, made by skilled carvers who not only worked faithfully in their own traditions, but were also capable on occasion of representing extraneous objects in their own style.

## New Floristic Studies

ATTENTION may be directed to two new and considerable floristic studies. C. Schweinfurth has added a further contribution on the "Orchids of Peru" (published in *Fieldiana: Botany*, **30**, No. 2; Chicago Natural History Museum, March 1959, 4 dollars 50 cents). The work is in the usual format for this series, and is mainly devoted to two considerable genera, namely *Pleurothallis* and *Epidendrum*. Ten other genera with a smaller number of species are also considered. All the available information has been used in preparing this volume, though some of the records are understandably still rather scanty. The publication is well illustrated by line drawings.

J. P. M. Bronan has made a further contribution to the "Flora of Tropical East Africa", the portion now published dealing with Leguminosae subfamily Mimosoideae (publ. Crown Agents for Oversea Governments and Administrations, London, May, 1959; price 12*s*.). The main features of the subfamily are set out together with a bibliographical commentary and there is an analytical key to the genera based on vegetative and fruit characters; there are also analytical keys to the species within individual genera. The text contains much useful descriptive matter dealing with distribution, habitat, etc., and is illustrated by line drawings.

## Royal Commission for the Exhibition of 1851

THE following awards have been made for 1959: *Senior Studentships:* D. W. Barnes (University of Oxford) for research in pure mathematics at Tübingen; P. J. Goodford (University of Oxford) for research in pharmacology at Oxford; A. V. Grimstone (University of Cambridge) for research in zoology at Cambridge; D. O. Hayward (Imperial College of Science and Technology) for research in physical chemistry at the Imperial College of Science and Technology; M. Wells (University of Cambridge) for research in physics at Cambridge. The Senior Studentships are of the value of £800–£900 a year and tenable ordinarily for two years. *Overseas Scholarships:* G. F. O. Langstroth (Dalhousie University) for research in physics at University College, London; A. J. McComb (University of Melbourne) for research in plant physiology at King's College, London; J. W. White (University of Sydney) for research in physical chemistry at Oxford; Miss S. G. Page (University of New Zealand) for research in biophysics at University College, London; A. Chisholm (University of New Zealand) for research in physics at Liverpool; M. H. Proctor (Trinity College, Dublin) for research in biochemistry at Cambridge; A. J. Ganguly (University of Delhi) for research in organic chemistry at the Imperial College of Science and Technology, London; M. Jameel (University of Karachi) for research in physics at Cambridge. The Overseas Scholarships are of the value of £550–£650 a year and tenable for two or three years.

## American Academy of Arts and Sciences: Foreign Honorary Members

THE American Academy of Arts and Sciences, at its 179th annual meeting on May 13, in Boston, elected 113 new Fellows from the United States, and 21 new Foreign Honorary Members as follows: Sir John Eccles, Australian National University, Canberra; Jean Brachet, Université libre de Bruxelles; Georges Braque, Paris; Albert Camus, Paris; Jean Leray, Collège de France, Paris; Max Born, Bad Pyrmont, Germany; George Keith Batchelor, Cambridge; Sir Isaiah Berlin, Oxford; Sir Lawrence Bragg, Royal Institution, London; Frank C. Francis, British Museum, London; Anna

Freud, London; David Keilin, Cambridge; P. B. Medawar, University College, London; Sir George White Pickering, Oxford; Ronald Syme, Oxford; Federico Chabod, Croce Institute, Naples; Hitoshi Kihara, National Institute of Genetics, Misima; Carlos Chavez, Mexico City; M. G. J. Minnaert, University of Utrecht; Alf A. Sommerfelt, University of Oslo; A. N. Kolmogorov, Academy of Sciences, Moscow.

At the same meeting Dr. Kirtley F. Mather, emeritus professor of geology, Harvard University, was re-elected president for another year.

## University News:

### Bristol

The following appointments have been made: Dr. J. T. Martin to a readership in chemistry of insecticides and fungicides. Lectureships have been conferred on Dr. E. W. Abel (inorganic chemistry), Dr. R. F. Burbridge (electrical engineering) and R. H. C. Penny (chemical pathology; veterinary).

### University College, Dublin

Dr. A. L. Kapoor of the National Chemical Laboratory, Poona, has been appointed an I.C.I. Fellow in Chemistry at University College, Dublin.

### Liverpool

The following appointments have been made to take effect from October 1: Dr. B. Collinge, reader in physics; Dr. P. M. Sheppard, reader in genetics. The following have been appointed to senior lectureships: Dr. A. K. Holliday, inorganic and physical chemistry; Dr. N. S. Jones, marine biology; Dr. T. M. Flett, pure mathematics; Dr. A. Ashmore, physics; Dr. H. D. Parbrook, physics-acoustics; Dr. V. H. Leck, electronic engineering; Dr. R. S. Benson and Dr. N. G. Calvert, mechanical engineering; Dr. F. T. W. Jordan, veterinary preventive medicine.

### University College of North Staffordshire

Dr. D. J. E. Ingram, at present reader in electronics in the University of Southampton, has been appointed professor of physics, to take effect from October 1.

### Nottingham

The following appointments have been made to take effect from October 1: Dr. K. J. Standley, to a readership in physics; Dr. G. E. Lamming, to a readership in animal physiology; Dr. M. Woodbine, to a readership in agricultural microbiology.

### Sheffield

The following appointments have been announced: Dr. D. E. Bourne, lecturer in applied mathematics; Dr. R. S. Duff, senior lecturer in medicine; I. E. Gillespie, lecturer in surgery; D. W. Warrell, lecturer in obstetrics and gynaecology; L. Grimshaw, lecturer in psychiatry.

### Swansea

Prof. F. Llewellyn Jones, professor of physics, University College of Swansea, has been appointed acting principal of the College until such time as a new principal takes office.

## Announcements

Dr. P. T. Haskell has been appointed deputy director of the Anti-Locust Research Centre, London, in succession to Dr. T. H. C. Taylor, who became director on the retirement recently of Dr. B. P. Uvarov (see *Nature*, **183**, 1160; 1959). Dr. Haskell has been at the Centre since 1955, and was previously lecturer in entomology in the Imperial College of Science and Technology, London. Most of his published work has been concerned with sensory physiology, especially the production and perception of sound in insects.

Dr. Carl F. Kossack, formerly head of the Department of Mathematics and Statistics at Purdue University, has joined the research organization of the International Business Machines Corporation at the Lamb Estate Research Center in the Town of Cortlandt, New York. Dr. Kossack is manager of the newly formed Statistics and Operations Research Department. Dr. Kossack gained his B.A. and M.S. degrees in mathematics at the University of California and his Ph.D. degree in mathematical statistics from the University of Michigan.

A joint meeting of the Challenger Society and representatives from the marine laboratories (Development Commissioners' scheme) will be held at the Guildhall, Conway, North Wales, on October 28 and 29. Further particulars of the meeting can be obtained from Dr. H. O. Bull, Dove Marine Laboratory, Cullercoats, Northumberland.

The U.S.S.R. Academy of Sciences began to publish in the Russian language in 1959 the following new journals (in brackets, the first figure indicates number of issues per annum; the second figure, the price in roubles per annum): *High Molecular Compounds* (12, 150); *The Geology of Ore Deposits* (6, 72); *Palæontological Journal* (4, 60); *Radiochemistry* (6, 72); *The Physics of the Solid Body* (12, 150); *Cytology* (6, 72); *Soviet Slavic Studies* (4, 50).

The Commonwealth Scientific and Industrial Research Organization of Australia has issued a pamphlet listing the Organization's Divisions and Sections, as at January 1, 1959, giving the address of each and its laboratories, and the names of the Officers-in-Charge. State Committees are also listed. Publications of the Organization, to December 31, 1958, including those of its predecessors, are listed in a separate pamphlet.

Volume XV of the Collected Papers of the Rowett Research Institute (Bucksburn: Rowett Research Institute, 1959) contains an account of work of the Institute, lists of members of its governing body and scientific staff and of 88 published papers, reprints of most of which are available, as well as a summary, by the Director of the Institute, of the contents of published papers of the Institute 1957–58 so arranged as to indicate the scope, continuity and integration of the research programme. Two subject reviews are also included: by J. J. Bullen on experimental reproduction of enterotoxæmia of sheep and by J. Duckworth on Institute research on the skeleton in lactation and growth.

The Department of Scientific and Industrial Research will shortly resume publication of its *Technical Digests*, last issued in 1957. Their object is to direct attention to useful ideas appearing in technical literature, and following an initial free distribution to industrial firms they will be available at an annual subscription of £3 3*s*. A reduction will be offered for supplies in quantity. The digests will be published monthly, each being printed on a separate sheet of paper.

# THE TWO CULTURES AND THE SCIENTIFIC REVOLUTION

SIR CHARLES P. SNOW'S Rede Lecture* for 1959 carries forward to a significant extent the old arguments concerning those subjects commonly spoken of as the humanities on one hand and the sciences on the other. The teaching of the history and sociology of science to arts students and the teaching of the history of art and literature to science students is better than nothing, though it is difficult to see how the history of science can make much impact without a knowledge of the methods and results of scientific investigation. The corpus of knowledge of all types is now so vast that it is foolish to look back to a Hellenic or Thomistic attempt at a synthesis.

Sir Charles Snow is both a literary man and a scientist and is able to see each side of the problem from the other. He seems to find the smug, self-contentment of many of the literary men more dangerous and irritating than the failure of some scientists to realize the implications of their own work in the broader field of human knowledge and aspiration. "Why do most writers take on social opinions which would have been thought distinctly uncivilized and démodé at the time of the Plantagenets? Wasn't that true of most of the famous twentieth-century writers? Yeats, Pound, Wyndham Lewis, nine out of ten of those who have dominated literary sensibility in our time—were they not only politically silly, but politically wicked? Didn't the influence of all they represent bring Auschwitz that much nearer?"

But it is ill-considered of scientists to judge writers on the evidence of the period 1914–50. Literature changes more slowly than science. It has not the same automatic corrective and so its misguided periods are longer.

"At one pole, the scientific culture really is a culture, not only in an intellectual but also in an anthropological sense. Its members need not always completely understand each other—biologists more often than not will have a pretty hazy idea of contemporary physics—but there are common attitudes, common standards and patterns of behaviour, common approaches and assumptions. This goes surprisingly wide and deep. It cuts across other mental patterns, such as those of religion or politics or class. . . . In their working, and in much of their emotional life, their attitudes are closer to other scientists who in religion or politics or class have the same labels as themselves. At the other pole, the spread of attitudes is wider. It is obvious that between the two, as one moves through intellectual society from the physicists to the literary intellectuals, there are all kinds of tones of feeling on the way. But I believe the pole of total incomprehension of science radiates its influence on all the rest. That total incomprehension gives, much more pervasively than we realize, living in it, an unscientific flavour to the whole 'traditional' culture, and that unscientific flavour is often, much more than we admit, on the point of turning anti-scientific . . . Once or twice I have been provoked (by the 'non-scientists') and have asked the company how many of them could describe the Second Law of Thermodynamics. The response was cold: it was also negative. Yet I was asking something which is the scientific equivalent of: Have you read any work of Shakespeare's?"

Little purpose is served by cataloguing the dismal ignorances in many arts people of the simplest, fundamental principles of science. "The separation between the scientists and non-scientists is much less bridgeable among the young than it was thirty years ago; then they managed a kind of frozen smile across the gulf. Now the politeness has gone and they just make faces. It is not only that the young scientists now feel that they are a part of a culture on the rise while the other is in retreat. It is also, to be brutal, that the young scientists know that with an indifferent degree they will get a comfortable job, while their contemporaries and counterparts in English or History will be lucky to earn 60 per cent as much. No young scientist of any talent would feel that he is not wanted or that his work is ridiculous as did the hero of Lucky Jim; and in fact some of the disgruntlement of Amis and his associates is the disgruntlement of the under-employed arts graduates."

Sir Charles insists that we should completely rethink our education. "Nearly everyone will agree that our school education is too specialized. But nearly everyone feels that it is outside the will of man to alter it. Other countries are as dissatisfied with their education as we are, but are not so resigned. Because of our intense specialization, alleged by schoolmasters to be dictated by the Oxford and Cambridge Scholarship examinations, we have set ourselves the task of producing a tiny élite—far smaller proportionately than in any comparable country—educated in one academic skill." Snow takes as an example the old Cambridge Tripos which seemed to be perfect in all respects save one. The one exception was—so the young creative mathematicians, such as Hardy and Littlewood, kept saying—that the training has no intellectual merit at all. They went a little further and said that the Tripos had killed serious mathematics in England stone dead for a hundred years.

While we are beginning, after many years, to understand the scientific and social implications of the industrial revolution and to understand the development in teaching technology in Germany in the nineteenth century and in the United States and U.S.S.R. in later years, we are still far from grasping the meaning of the scientific revolution, by which is meant the transformations made in industry and its effects because of electronics, atomic energy, automation and modern forms of machine tools.

We have failed to keep pace with the new scientific revolution—"roughly if we compare like with like and put scientists and engineers together we are training at a professional level per head of the population one Englishman to every one and a half Americans to every two and a half Russians. In Russia the gap between the cultures does not seem to be as wide as it is with us. If one reads contemporary Soviet novels, for example, one finds that their novelists can assume in their readers—as we cannot

* Published by the Cambridge University Press.

—at least a rudimentary acquaintance with what industry is all about". The latest figures of graduates trained per year (scientists and engineers combined) are roughly United Kingdom, 13,000; United States, 65,000; U.S.S.R., 130,000. One-third of Russian graduates in engineering are women. "It is one of our major follies that we do not in reality regard women as suitable for scientific careers. We thus divide our pool of potential talent by two."

"We are left with a population twice as large as we can grow food for, so that we are always going to be *au fond* more anxious than France or Sweden, and with very little in the way of natural resources—by the standard of the great world powers, with nothing. The only real assets we have, in fact, are our wits. Those have served us pretty well, in two ways. We have a good deal of cunning, native or acquired, in the arts of getting on among ourselves: that is a strength, and we have been inventive and creative, possibly out of proportion to our numbers. Given these two assets, and they are our only ones, it should have been for us to understand the scientific revolution first, to educate ourselves to the limit and give a lead. In some fields, like atomic energy, we have done better than anyone could have predicted. Within the pattern, the rigid and crystallized pattern of our education and of the two cultures, we have been trying moderately hard to adjust ourselves. The historical warnings are all there. For instance, the Venetian Republic in its last half-century was guided by patriot men, who had immense political skill, who knew that the current of history had begun to flow against them. They were fond of the comfortable pattern of their life, just as we are fond of ours. They never found the will to break it."

There is yet another danger. The large masses of poor in the undeveloped countries will not allow themselves to live for ever in a world in which large sections have become rich through industry. A new missionary spirit, both human and technical, is essential.

"Closing the gap between our cultures is a necessity in the most abstract intellectual sense, as well as in the most practical. When those two senses have grown apart, then no society is going to be able to think with wisdom. For the sake of the intellectual life, for the sake of this country's special danger, for the sake of the western society living precariously rich among the poor, for the sake of the poor who need not be poor if there is intelligence in the world, it is obligatory for us and the Americans and the whole West to look at our education with fresh eyes. This is one of the cases where we and the Americans have the most to learn from each other. We have each a good deal to learn from the Russians, if we are not too proud. Incidentally, the Russians have a good deal to learn from us too." W. L. Sumner

# COAL SCIENCE

THE third biennial International Conference on Coal Science was held at Valkenburg, in the Netherlands, during April 27–30. The Municipality kindly allowed the Conference to be held in the Municipal Theatre, and the greatest hospitality and interest were shown throughout by the burgomaster, F. A. A. H. Breekpot.

On this occasion the number of participating countries increased to fourteen, newcomers being East Germany, Czechoslovakia, Australia and India. Authors submitting papers were required to complete a form indicating, in telegraphic style, their main new conclusions and results, the methods and observations from which these were deduced, and any special limitations or assumptions involved in their interpretation. This information was found by the organizers to be more helpful than the conventional summary (or on occasions the paper itself) in deciding whether a paper was acceptable; it was also useful in evaluating the conclusions of the Conference and the interrelation of papers. The proceedings of this Conference will not be published as a whole; but papers will be submitted by their authors to journals of their own choosing.

Discussion was unusually lively and fruitful, and a large proportion of novel work was presented. In several cases widely accepted ideas were apparently undermined, though it would be premature to assess the importance of the new evidence. For example, a suggestion arose, from the work of S. Ergun and I. Wender on the X-ray scattering of vitrinites reduced with lithium in ethylene diamine, that partially reduced aromatic and/or alicyclic molecules may lead to reflexions in the angular region where the (10) and (11) reflexions of aromatic molecules occur. This awaits direct experimental test, but if correct it may throw doubt upon the derivation of aromatic layer sizes in coal, for example, by Hirsch and Diamond. Ergun and Wender also demonstrated an increase of layer-spacing on reduction, even in graphite.

Similarly, an interpretation of data obtained on coal by high-resolution magnetic resonance spectroscopy, by J. K. Brown, W. R. Ladner and N. Sheppard, yielded a structural distribution of non-aromatic carbon atoms which may prove incompatible with the present consensus of opinion on the chemical structure of coal. As a third example, R. L. Bond and D. H. T. Spencer illustrated the inadequacy of existing interpretations of sorption data obtained with inert gases on coal and of heats of wetting of coal in polar liquids; a question thought by many to have been settled, at least in principle.

These, and the other thirty-three papers presented, were grouped under four heads, as follows.

## Chemical Reactions of Coal

E. S. Hammack, H. G. Davis and F. B. Brown estimated the content of phenolic hydroxyl in vitrains by titration with sodium aminoethoxide in ethylene diamine, by trimethylsylylation and by acetylation. They found reasonable agreement between these methods and noted the importance of particle size. Acetylation was studied by S. Delavarenne, A. Halleux and H. Tschamler, who also confirmed the presence of quinone groups in coal extracts, by reductive acetylation and reduction with copper/hydrogen sulphide. They observed the corresponding changes in the infra-red spectra and showed that the

reduction was to a large extent reversible. In both papers, tests of the quantitative accuracy of the methods by experiments on model compounds such as quinones were reported. Evidence for the presence of thioether groups in coals to the extent of 15–90 per cent of the organic sulphur content was put forward by L. Wnękowska. The difficulty of combining all known observations on the chemical and physical nature of coal, together with the elementary analysis, in a structural model was emphasized by P. H. Given, who by comparison of data and experiments with atomic models had been able to find only one satisfactory pattern of structure: the essential feature of this was that any pair of aromatic fused ring clusters is bound together by *two* methylene bridges.

E. J. Greenhow and J. W. Smith showed that pitch can be regarded as a solution of phenolic or basic compounds of medium molecular weight in a relatively non-polar solvent; results suggested that the physical properties were influenced by intermolecular association.

There were three papers on chromatographic separation of oxidation products of coal: by F. Micheel, J. E. Germain and F. Valadon, and G. J. Lawson and S. G. Ward. The first author had isolated small yields of aniline, anthracene, substituted anthraquinone, tetraphene and fluoranthene from air- or nitric acid-oxidized coals; the second group had identified various benzene polycarboxylic acids, and the third, malonic, succinic and *l*-tartaric acids. S. Landa had studied hydrogenation of model substances, montan wax and humic acids extracted from brown-coal, with tungsten and molybdenum sulphide catalysts. Hydrocarbons with two to three fused aromatic rings and side-chains were found among the products from treatment of the humic acids.

## Ultra-fine Structure of Coals and Chars

M. Węclewska discussed the influence of sorption on coals of organic vapours on their elasticity, and the consequent dimensional changes. S. Ergun, W. F. Donaldson and I. Breger showed that apparent changes in rank induced in coals by α-particles originating from natural impregnation by uranium differed from those caused by normal coalification processes.

J. L. Soulé and S. Durif demonstrated, by measurements of small-angle X-ray scattering, the presence in a wide range of coals of pores of sizes 22–25 A. and the variation of this characteristic with temperature of carbonization; they also deduced absolute values of specific internal surface. In the carbonization temperature range 500–700° C. these surface areas agreed with those estimated by P. Chiche and S. Prégermain from adsorption of water and methanol vapours.

Sorption of pyridine by carbonization products, their solubility in it and their swelling in various organic liquids, were studied by A. Ladam and P. Payen. Important changes were indicated in the region 400–500° C. S. J. Gregg and M. I. Pope had studied the effect, on the electrical conductivity of coal artefacts, of pressure and of exposure to atmospheres of various humidities. J. J. Kipling and R. B. Wilson, from a study of the sorption of gases and vapours on chars prepared by carbonizing synthetic polymers, demonstrated the presence of microcapillaries of 5–10 A. diameter, molecular sieve properties of the structure, and the effect of steam activation in opening up this structure. A. Cameron and W. O. Stacey combined measurements of internal surface area, apparent density and measurement of internal volume with the porosimeter to demonstrate the presence of two distinct pore systems in chars prepared from brown-coals. The presence in 600–900° C. chars of cavities of some 25 A. linked by passages less than 10 A. wide was inferred.

## Spectroscopy of Coals

There were two papers on mass spectroscopy. One, by J. C. Robb and H. W. Holden, was concerned with the volatile products either volatilized or liberated by pyrolysis when coal was heated in the ion source. Interesting identifications in relation to temperature range were made. The other, by R. I. Reed and W. Snedden, was chiefly concerned with the fragments produced from aromatic compounds and thus widened the background available for interpreting results with coals. Electron magnetic resonance spectroscopy had been used to study coals in course of carbonization under vacuum, by J. Smidt and D. W. van Krevelen. In addition, the time of relaxation $T_1$ was measured as a function of temperature. J. Uebersfeld had applied combined nuclear and electron magnetic resonance measurements and found evidence of interaction between protons and free radicals in coals of low rank, exchange interaction in those of high rank. Light spectroscopy was represented in two papers, by R. A. Durie and J. Szewczyk and by S. Ergun and J. T. MacCartney. The first was concerned with a long wave-length absorption edge which moves into the infra-red with increasing temperature of pyrolysis. The magnitude of a corresponding energy gap in the structure was discussed in relation to the graph of polynuclear aromatic structures. In the other paper an attempt was made to establish specific reflectance of coals as a significant structural parameter, related to the hydrogen/carbon ratio and to the diamond and graphite structures as extremes.

## Carbonization

C. Kröger, R. Brücker, M. Klatt and E. Bade had studied the maceral species exinite and vitrinite by pyrolysis under high vacuum. They discussed the origin of the liquid and gaseous products determined. A. M. Wandless and G. W. Fenton described coking tests, on coals of 84, 85 and 87 per cent carbon content, that indicated a beneficial effect on the coke strength of the maceral inertinite as a constituent in the coal blend at the two lower levels of rank. D. W. van Krevelen, F. J. Huntjens and A. H. Wilms presented a thorough study of strength of coke in relation to particle size of coal carbonized. A. D. Dainton, W. G. Kaye and J. W. Phillips described the effect of macerals on volume changes in briquettes during carbonization. A. F. Boyer and P. Payen threw doubt on the interpretation of an apparent exothermic peak found in the differential thermal analysis of certain coals; they considered this to result from an increase of conductivity due to agglutination. Finally in this series there was an outstanding paper by P. M. J. Wolfs, D. W. van Krevelen and H. I. Waterman on the carbonization of synthetic polymers containing aromatic units linked by methylene bridges labelled with radioactive carbon; their fate on pyrolysis could be readily followed. The course of chemical decomposition was thus related to other measurements on the polymer, for example, in a dilatometer test and a thermobalance.

M. F. Kessler and V. Večeřiková reported a variety of physical and physicochemical measurements on cokes and related them to hardness and reactivity. J. H. Ehretsmann and R. C. Seymour had studied the gaseous products and thermochemistry of the formation and decomposition of surface oxides on charcoals. H. Guérin and M. Bastick demonstrated the importance of microporosity during gasification of coke between 800° and 1,000° C. C. Heuchamps, L. Bonnetain, X. Duval and M. Letort showed that the apparent energy of activation of the combustion of graphite varied with temperature and extent of combustion, and was a complex function of heterogeneity, porous structure, impurities and reaction mechanism.

It is appropriate after three successive occasions when the burden of organization has largely been borne by the Dutch State Mines to add an appreciation of the magnificent arrangements made by the scientific and administrative staff of that body. The never failing resourcefulness of Mr. W. J. R. Berkx in all aspects is especially worthy of mention. The moving spirit behind these conferences has been the president, Prof. D. W. van Krevelen, and it must be recorded with regret that he has now resigned the presidency in view of his pending transfer from the coal to the plastics industry. To mark the occasion, tributes were paid at the end of the Conference by R. Loison, I. G. C. Dryden and M.-Th. Mackowsky on behalf of the French-, English- and German-speaking sections of the audience. Prof. van Krevelen's impact on coal science has occurred only within the past ten years ; yet, owing to the original and prodigious output of his research teams, he has left nothing unchanged. The Conference wished him every success in his new field of endeavour.

I. G. C. Dryden

# CURRENT RESEARCH ON NOISE

IT is only within recent years that acoustic noise, once studied simply for its effect on hearing and annoyance, has proved important in physical systems. Noise has long been a source of annoyance ; its effects on working efficiency have been clear when it has interfered with speech communication, less clear in causing fatigue ; and for years it has been realized that continued excessive noise can cause permanent deterioration of hearing—'boilermaker's deafness' has long been known.

These effects are still with us, in intensified form, since with the increased power of machines to-day, noise—as a by-product of this power—is increasing also. For this reason, the physical aspects of noise have come to the fore. The intense fluctuating pressures in the noise field near a jet engine can fatigue and fracture the structure of the aircraft. The forces on a missile flying through rough air may consist largely of random constituents, which need to be analysed in some detail to determine whether any frequencies are present which may damage the equipment in the missile. Such analysis is, of course, similar to that used in many other fields, such as radar ; and thus acoustic noise problems find their counterparts in other studies.

These different aspects of noise were explored recently in two symposia held by the Acoustics Group of the Physical Society. The first, "Recent Studies of Noise Problems", was held at the Imperial College of Science and Technology, London, on March 24, with four papers on psycho-acoustic problems, and two on physical acoustics.

## Human Response to Noise

The general effect of the first group of papers, curiously, was that work in many aspects of the subject culminated some five years ago in the United States, and little advance has been made since. This was particularly true of the account of noise and hearing loss given by Prof. W. Burns (Charing Cross Hospital Medical School). This subject is still heavily in debt to the classic report (*Z24-X-2*) of the American Standards Association, and little more has been learnt about safe noise-levels. However, work is continuing on the effect of age on normal hearing ; this will give a better 'base-line' for the estimation of hearing damage, and is a subject on which the American report was not very satisfactory.

Procedures for evaluation of the loudness of a complex sound, however, have progressed very little in any country. This was discussed by Mr. N. Fleming and Mr. D. W. Robinson (National Physical Laboratory). The National Physical Laboratory equal-loudness contours remain unchallenged, but they are for pure tones only. The various ways of dividing and weighting the spectrum, in order to calculate loudness, do not seem very productive ; while annoyance is a factor even harder to measure objectively.

Much of this difficulty may be due to the fact that pure tones have in the past been considered of major importance. But as speech, and even music, are in effect successions of transients, many noises should be considered in the same way ; and a study of the aural effects of transients may be a more fruitful approach.

The point was, however, made by Mr. Fleming that the multiplicity of criteria in American psycho-acoustic studies may not be needed. He pointed out the equivalence between the 'equal-annoyance' contours for community noise, and the readings of representative noises on the *A* weighting of the sound-level meter. The *A* weighting is a valuable one for many approximate loudness studies ; it is frequently used on the European continent in traffic-noise problems.

Further points on community reaction to noise were dealt with by Mr. H. J. Purkis (Building Research Station). The techniques for evaluating the annoyance of different types of noise, developed in America some years ago, still appear generally valid, although some alterations to suit the differing susceptibilities of English communities are needed. It is hoped to collate several case histories of noise annoyance, with the view of obtaining more applicable data.

Dr. D. E. Broadbent (Medical Research Council) spoke on the effects of noise on working efficiency. This is a field in which it is most difficult to obtain conclusive results. A pattern is, however, beginning to emerge, connected with the effect of noise in interrupting attention ; noise often seems to interfere with tasks necessitating short-term memory. An overall level of 90 db. appears critical, and it is found

that, whatever may be the effect on efficiency at work, noise increases, for example, wastage rate, and number of mistakes.

### Physical Acoustics

Two papers on physical noise problems were presented. Mr. D. M. A. Mercer (University of Southampton) dealt with the difficulties inherent in obtaining accurate measurements of aircraft noise. Such factors as ground reflexions, size and behaviour of source can cause errors of a few decibels; comparisons of, for example, the performance of jet silencing devices may be quite misleading if these precautions are not taken.

Diesel engine noise was discussed by Dr. A. E. W. Austen and Dr. D. Priede (C.A.V., Ltd.). Speed and swept volume have been related to sound output; there is little variation with load, due to the characteristics of the diesel engine. A quite thorough survey has been made of the vibration of different surfaces of the engine and their effects as noise producers, and sound-levels calculated from these are consistent with measured noise-levels.

### Aspects of Noise Analysis

The second symposium, on "New Techniques in the Analysis of Noise and Vibration", was held in the Physics Department of the University of Southampton, as a joint meeting with the Institute of Physics. The attendance of about 150, drawn from diverse fields of physics, engineering and electronics, indicated the considerable interest in the analysis of 'noise in the widest sense'. The emphasis was on new and projected techniques; standard bandpass filtering methods received little attention.

The meeting was opened by Mr. R. A. Eades (Signals Research and Development Establishment, Christchurch), who described the speech spectrograph, an instrument capable of examining and analysing short samples of sounds, and presenting the analysis in a manner similar to that of the visible-speech techniques. Mr. Eades made the important point that, in much of this work, bandpass filters with sharp cut-offs are not desirable, due to the 'ringing' they give; the lowered frequency discrimination of a filter of Gaussian or tuned-circuit characteristics is amply repaid by freedom from ringing.

Mr. G. J. Herring (Royal Aircraft Establishment, Farnborough) described an analogue-to-digital converter which converted a fluctuating voltage into an input suitable for a *Pegasus* digital computer. The computer could then be used to make an analysis of the input wave-form. This method was developed because purely analogue methods had proved unsatisfactory. This paper, and the previous one, led to considerable discussion; it was clear that the problem of analysis of random wave-forms was important to many workers. One point, however, was that the distinction between wave-forms with and without a periodic component—a difference important to the statistician—was not always made clear; with consequences perhaps of unnecessary difficulties in many analyses.

Mr. D. M. A. Mercer (University of Southampton) emphasized the practical point that, in any analysis, frequency discrimination, stability of estimates and ease of computing were all linked, and could not all be maximized at once; great care was needed in the planning stages of an analysis. Cross-correlation techniques were often able to give results unobtainable by straightforward methods.

Dr. G. M. Jenkins (Imperial College of Science and Technology, London) discussed the statistical implications of obtaining a spectrum via an autocorrelogram. It is desirable to examine the autocorrelogram itself before the Fourier transform process; in any event, some modification to it is often desirable, to obtain a more stable spectrum, but in addition much information is often available at this stage; and different applicable statistical criteria were described.

The ensuing discussion demonstrated much interest in the use of axis-crossings of a random function. Presumably there is a major theoretical breakthrough to be looked for on this topic; but apart from this, theoretical and experimental studies demonstrate that axis-crossings carry most of the information of a wave-form (apart from magnitude, of course). The limitations of the method, however, are not yet known.

The concluding impression of the symposium appeared to be twofold: first, that 'noise in the widest sense' is a subject of considerable interest, and secondly, that there often appeared to be a considerable gap between the theoretical studies, and the work of those concerned with practical noise and vibration analysis. This leads, at best, to correct analyses obtained with unnecessary difficulty, and at worst, analyses which are meaningless. Closing of this gap would be of benefit to all concerned.

DERWENT M. A. MERCER

## BIOSYNTHESIS AND SECRETION OF ADRENOCORTICAL STEROIDS

AT a symposium held on February 14, the Biochemical Society gathered together a group of investigators who described their own work and that of others on various aspects of the biogenesis of the adrenocortical hormones. In an introductory paper, I. E. Bush described the chemical nature of the steroids under discussion and the application of various types of chromatographic procedures to the separation and identification of these substances. An important feature of steroid chromatography is the very large number of possible compounds and the danger of wrong identification if chromatographic properties are relied upon to the exclusion of others. This danger may be diminished by the use of numerous microchemical and spectrophotometric techniques. The former include acetylation or oxidation (notably the removal of the C-17 side chain) on the 10–20 μgm. scale, followed by observation of the chromatographic behaviour of the product on paper. Spots of steroid on paper give various well-known colour and very sensitive fluorimetric reactions. On the physical side a wide range of correlations between structure and absorption bands in the infra-red region has been established. Ultra-violet absorption spectra in sulphuric acid and alcoholic alkali provide useful information which, in conjunction with paper chromatography and comparison with reference substances, may provide identifications almost as reliable

as those from infra-red spectra. New work in Bush's laboratory has shown that useful generalizations about the behaviour of steroids in partition systems can be made using Martin's theory and the $R_M$ function of Bate-Smith and Westall. For this theoretical approach Bush has collected numerous $R_F$ values from the literature. Not all will agree with him on the reliability of these values. The chromatogram tank is not yet a precision instrument.

J. K. Grant then reviewed present knowledge of the biosynthesis of adrenocortical steroids. Here there are at least three questions to be answered. What is the nature of the secretion of the adrenal cortex, what sequences of reactions are involved in the syntheses of the steroids in the cortical cells and what are the mechanisms of these reactions? Complete answers cannot be given. The analysis of adrenal venous blood collected from animals and from human subjects undergoing adrenalectomy has provided direct evidence of the nature of the steroids secreted by the adrenal gland. Hydrocortisone, corticosterone and aldosterone are the principal $C_{21}$ compounds found. The last of these, and a variety of others of less physiological importance, are present in trace amounts. There is indirect evidence that the adrenal cortex in man secretes a substance which, in contrast with aldosterone, promotes the excretion of sodium. A new steroid (3β : 16α-dihydroxy-5α-pregnan-20-one), which produces this effect in rats, has recently been isolated by Wettstein from the adrenal glands of swine. There is also evidence that the adrenal gland secretes androgenic and œstrogenic steroids.

Application of a variety of techniques has clearly established that adrenocortical steroids may be formed in the gland from acetate or cholesterol by formation and subsequent hydroxylation of progesterone. An outstanding question of major interest is the possibility of alternative pathways to those originally proposed. These alternatives are concerned with the order in which the hydroxylations of the key intermediate progesterone proceed, with the possibility that cholesterol is not an obligatory intermediate in hormone formation, and with the direct transformation of cholesterol to $C_{19}$ steroids. Hydroxylations commonly occur at C-17, C-21 and C-11β in that order. It has been shown beyond reasonable doubt that a C-21 hydroxy steroid cannot undergo hydroxylation at C-17. Grant has, however, demonstrated that 11β-hydroxylation of progesterone can proceed in good yield. Hydrocortisone may then be formed from 11β-hydroxyprogesterone but at rates too slow to be of significance in normal biosynthesis. This alternative pathway may, however, be important in pathological states. Despite much suggestive evidence and numerous experiments with isotopically labelled compounds, it has not been possible to establish firmly the existence of alternative pathways from acetate to adrenocortical hormones which do not involve cholesterol. When such pathways appear to have been demonstrated, alternative explanations of the results have been possible if the existence of different metabolic pools of adrenal cholesterol is assumed. In other cases the results are unacceptable since the comparisons between the specific activities of precursors and products have been made at one point in time and not on a kinetic basis known to be essential in order to avoid misinterpretation of results. Despite intensive study, the mechanism of steroid hydroxylation has not been elucidated. Molecular oxygen and reduced triphosphopyridine nucleotide are essential. It has not been possible to confirm that a transhydrogenase is involved in the formation of reduced triphosphopyridine nucleotide. It has been suggested that the reduced nucleotide is required for the reduction of an oxygen activating or transferring enzyme containing a heavy metal, but proof is lacking. There are, however, indications that the 11β-hydroxylating enzyme system located in the mitochondria is complex. The observation that 2-methyl-1 : 2-bis-(3-pyridyl)-1-propanone (*SU*.4885) appears to be a specific inhibitor of 11β-hydroxylation *in vivo* and *in vitro* has aroused considerable interest.

Adrenal glands of the rat, ox and man have been most extensively studied. The anatomy and histology of these glands were described by T. Symington. He directed attention to two features which have not until recently received adequate attention from biochemists: the unusual musculature of the adrenal veins which may, by restricting blood flow through the gland, influence steroid biosynthesis, and the marked differences in histology between the ruminant and non-ruminant adrenal cortex. The fasciculata and reticularis zones, readily distinguished in non-ruminants, are not distinguished in the ruminant. In the ox, which is typical of the ruminants, a broad zone of 'compact' cells fills the cortex from the medulla to the prominent glomerulosa beneath the capsule. In man, the fasciculata zone of variable width is filled with lipid-laden 'clear' cells and lies between the reticularis zone of 'compact' cells and the glomerulosa, which is very irregular, forming islands under the capsule so that glomerulosa cells are absent from some sections. The ox adrenal gland is for this reason a more satisfactory object than the human gland for the *in vitro* study of the synthesis of aldosterone which appears to occur in glomerulosa cells. The morphology and histochemistry of 'clear' and 'compact' cells and the influence of corticotrophin thereon have been studied in Symington's laboratory using glands removed surgically in two stages for the treatment of breast cancer. The use of these fresh glands has also permitted the study of enzyme reactions involved in steroid biosynthesis. These biochemical studies and in addition the results of analysis of adrenal venous blood obtained at the time of adrenalectomy have been correlated with the morphological and histochemical observations. It would appear from the results obtained that 'clear' cells of the fasciculata zone may function as stores of hormone precursor, and that the 'compact' cells of the reticularis zone, far from being the senescent cells described by others, may be the site of active hormone biosynthesis. 'Clear' cells are converted to 'compact' cells under the influence of corticotrophin, which stimulates corticosteroid secretion, influencing various steps in the sequence of biosynthetic reactions. Studies with the electron microscope in Glasgow have revealed important differences between the 'clear' and 'compact' cells, the most striking features of which are the microvilli, which form extensions of the cell membrane running out into the intercellular spaces. The function of the microvilli is unknown, but it is interesting to speculate that they may be concerned with secretory processes.

P. J. Ayres described the relation of steroid secretion to the histological zones of the adrenal cortex, with particular reference to the evidence which he has obtained in collaboration with Dr. and Mrs. Tait that aldosterone is synthesized and secreted

by the cells of the glomerulosa zone. This work developed out of early observations on the rat ; these supported the view that a steroid active in electrolyte metabolism was secreted by the glomerulosa zone, relatively independent of the pituitary but influenced by electrolyte intake. Further indirect evidence followed the isolation of the steroid concerned, aldosterone, from whole adrenal extract by the Taits in 1952. It was possible to show that aldosterone secretion, but not that of corticosterone, is diminished in rats on a low-potassium diet. Hypophysectomy has a much more pronounced effect on corticosterone than on aldosterone secretion in the rat. After this operation in dogs, the fasciculata-reticularis zone of the adrenal gland atrophies and the secretion of hydrocortisone and corticosterone falls ; by contrast the glomerulosa zone and the secretion of aldosterone are unaffected. Direct evidence for the preferential production of aldosterone in the glomerulosa zone was obtained by the Taits and their collaborators by incubation of adrenal capsule strippings, to which glomerulosa cells were adhering, and of tissue from the underlying zone. In the ox the 17-hydroxylating enzyme system appears to be in the zone lying under the glomerulosa only, whereas the 18-oxidase system necessary for aldosterone synthesis is confined to the glomerulosa zone.

A remarkable feature of the adrenal cortex is its ability to synthesize all known types of steroid hormones. R. V. Short discussed the biosynthesis and secretion of sex hormones by the adrenal gland. In his laboratory, methods have been developed for the separation and determination of the major and some of the minor steroid constituents of adrenal venous blood. These have been applied to the analyses of blood collected from human subjects by A. P. M. Forrest in Glasgow, and from adult, newborn and fœtal domestic animals by Dr. Short's collaborators in Cambridge. Progesterone has been found in adrenal blood from women, cows, sheep and pigs in concentrations higher than in the peripheral blood. 17α-Hydroxyprogesterone, a steroid which is sixty times as active as progesterone in some biological assays, has been detected in the adrenal venous blood of women and cows. The interesting observation has been made that young calves secrete 20α-hydroxypregn-4-en-3-one. This disappears from the secretion some time after birth and is replaced by the 20β-epimer in the adult animal. The significance of this is unknown. There is good indirect evidence for the secretion of œstrogens by the adrenal cortex, but they must be present in human adrenal venous blood in amounts too small for detection by present methods. Four androgens have been reported in adrenal-vein blood in man. Dehydro*epi*androsterone is probably exclusively of adrenal origin, but its precursors are still uncertain. Androstenedione is undoubtedly the most biologically active adrenal androgen and it may be present in adrenal venous blood in relatively high concentration. It is remarkable that androstenedione and dehydro*epi*androsterone cannot be detected in even large samples of adrenal venous blood in cattle. 11β-Hydroxyandrostenedione is probably present in the adrenal venous blood of the cow and sheep as well as in that of the rat and cat, but this compound, in common with the other known 11-oxy $C_{19}$ steroids, seems to possess little biological activity. These observations may be of considerable importance in veterinary medicine and cast doubts on the reports of adrenal virilism in cattle.

The amount of corticosterone and hydrocortisone secreted by the adrenal cortex is controlled by corticotrophin from the anterior lobe of the pituitary. How the pituitary obtains the information necessary to enable it to adjust the activity of the adrenal cortex to the body's requirements is a fascinating question which has recently attracted much attention. Knowledge of this matter was reviewed by Marthe Vogt. Few histologists consider the nerve supply to the adenohypophysis adequate for the control of corticotrophin secretion, and this control is therefore assumed to be humoral. It has been shown that substances such as adrenaline, which may be carried by the systemic blood, can promote the release of corticotrophin by the pituitary. There is also much evidence in support of the view that a stressing stimulus results in nervous activity in the hypothalamus which elaborates a 'corticotrophin-releasing factor'. This is carried by a 'portal' circulation to the anterior lobe, where it stimulates corticotrophin secretion. The original suggestion that the corticotrophin-releasing factor is identical with vasopressin has been modified by the independent observations of Saffran and Guillemin, who now propose that it is a polypeptide similar to but different from vasopressin. The purified material is active *in vitro* in nanogram amounts, and microgram quantities produce a similar effect on the rat pituitary stores of corticotrophin or on blood corticosteroids as severe stress. The active material contains seven of the eight amino-acids of lysine-vasopressin and, in addition, serine and histidine. Dr. Vogt also discussed the nature of stimuli which activate or inhibit hypothalamic activity. The level of corticosteroids in blood is now regarded as a contributing, but not the sole, factor accounting for the control of corticotrophin secretion in stress. Recent work suggests that the suppressing effect of high doses of corticosteroids may be on the hypothalamus rather than on the pituitary. In addition to lack of corticosteroid in the blood, afferent nervous impulses, especially if they elicit pain or worry, stimulate the release of corticotrophin, but the nature of the stimulus or stimuli which act in other forms of stress is unknown. General depressants of the brain inhibit the release of corticotrophin.

Dr. Vogt also reviewed knowledge of the control of secretion of aldosterone. This secretion is less influenced by corticotrophin than that of the glucocorticoids. Farrell has, however, recently found that certain preparations of corticotrophin increased aldosterone secretion in the decerebrate, hypophysectomized dog, but satisfactory evidence for the existence of a specific pituitary hormone which influences aldosterone secretion is lacking. The role of such a hormone would be limited, since increased aldosterone secretion in the hypophysectomized dog has been obtained with a variety of stimuli. Experiments in Dr. Vogt's laboratory have demonstrated the rapid and reversible response of aldosterone secretion in the dog to expansion and contraction of the intravascular volume. Infusion of blood decreases, whereas hæmorrhage increases, the secretion of aldosterone. The former effect was less easily elicited than the latter.

In the final paper, N. Saba dealt with the mode of action of corticotrophin from the biochemical point of view. He referred to the observations of Hechter that corticotrophin acts on the conversion of cholesterol to pregnenolone and the later independent work of Heard and Grant on the stimulation of

11-hydroxylation by the trophic hormone. Saba's results obtained in collaboration with Hechter suggest that corticotrophin influences the spatial relationships of enzymes involved in the biosynthesis of adrenocortical steroids. More recent work by others has revealed an effect of corticotrophin on enzyme systems which effects the reduction of triphosphopyridine nucleotide. The requirement for reduced triphosphopyridine nucleotide for steroid hydroxylation has already been referred to. If this is an important effect of corticotrophin it is difficult to relate it to the specificity of action of this hormone.

J. K. Grant

## EDUCATION IN THE UNITED STATES (1957–58)

EDUCATION in the United States of America is based on three fundamental concepts: that the primary responsibility for public education rests with the States; that every person has an equal right to educational opportunities; and that educated citizens are essential to freedom and human welfare. Working within these concepts during 1957–58 the United States made further progress towards its goal of improving education for all*.

Evidence of progress is found in the increasing educational attainment of the population. The number of school years completed by the average adult 25 years of age and over increased from 9·3 in 1950 to 10·6 years in 1957. The group 25–29 years had completed 12·3 years of schooling, while the group older than 65 had completed only 8·3 years.

Although education is a State responsibility, no State administers its schools directly. Laws have been enacted in each State dividing the territory of the State into local school administrative units, commonly termed school districts. The powers of local district school boards to establish and maintain schools are prescribed by State law, but permit exercise of local initiative in exceeding minimum educational standards required by the State. Each State has its own department of education, which exercises controls and provides specialized services to assist local school districts in conducting the State programme of education.

At the beginning of the school year there were 1,152,500 instruction rooms in full-time public elementary and secondary schools; an additional 142,300 rooms were needed to relieve over-crowding and to replace unsatisfactory facilities. About 70,500 instruction rooms were scheduled to be built during the year. About 61,000 rooms will be needed to accommodate next year's enrolment increase and to replace rooms that will probably be abandoned.

Almost one-third of all pupils enrolled in the public elementary and secondary schools are transported to and from school at public expense. During 1955–56, more than 10 million pupils were transported. In most States pupils must live 1½ miles or more from the school to be eligible for transportation for which the State helps to pay the cost.

Institutions of higher education classified by type of support and control are of two general types — publicly controlled and privately controlled. One-third of the approximately 1,900 higher institutions are publicly controlled and supported by public or government agencies; two-thirds are privately controlled and supported by individuals or ecclesiastic, philanthropic and other groups. The State exercises little control over institutions of higher education, even those supported by public funds, and consequently both types of institution operate with a high degree of autonomy.

In 1957–58 expenditures for education in public elementary and secondary schools and in higher institutions totalled 20,000 million dollars, which was 5·5 per cent of the 1957 total national income of 358,000 million dollars. Funds to cover expenditure in public schools were provided by Federal, State and local governments.

For the fiscal year 1958 the Federal Government appropriated approximately 2,000 million dollars for educational purposes. The total included funds administered by the Office of Education and was distributed as follows: Office of Education, 7,000,000 dollars; vocational education, 40,888,412 dollars; higher education, 5,051,000 dollars; school construction and maintenance, 225,650,000 dollars; and library services, 5,000,000 dollars.

Since the States have primary responsibility for public education in the elementary and secondary schools they provide funds and authorize local school districts to provide local tax-funds for public schools. In the 1957–58 school year it is estimated that of the total revenue for public schools the Federal Government provided 4 per cent; the States, 41 per cent; and local districts, 55 per cent. In recent years the percentage from Federal and States funds has been increasing slightly.

As much as 93 per cent of local educational revenue is obtained from property taxes. Local communities use the property tax to secure funds for current operating expenses and for school construction. Some local districts also levy non-property taxes for schools, including local *per capita* taxes and taxes on wages, sales and amusements. Non-property taxes for schools produce 7 per cent of local revenue.

Total expenditure per pupil in 1957–58 averaged 431 dollars, an increase of about 7 per cent over the amount in 1955–56.

Tax revenues supply most of the funds for public institutions; private donations, student tuition and endowment supply most of the funds for private institutions. The 1957 budgets for both private and public institutions for educational and general expenditures, excluding auxiliary services, student aid and plant expansion, totalled 3,200 million dollars. Of this, students paid 950 million dollars as tuition; earnings on endowment provided 150 million dollars; private gifts, 250 million dollars.

The organization of public schools is determined by State and local authorities, but generally the basic 12-year programme is organized as an 8-year elementary and a 4-year secondary programme or a 6-year elementary and a 6-year secondary programme. Typically a 6-year secondary programme is divided into a 3-year junior and a 3-year senior high school. The most common type of school is one

* Progress of Public Education in the U.S.A., 1957–58. (Washington, D.C.: Gov. Printing Office; available also from H.M. Stationery Office, London.)

attended by almost all children of school age in the community, regardless of social or economic status, sex or vocational aim. On all levels schools vary greatly in size, from one-room rural schools to large urban schools enrolling several thousand students. Improvement of the school district organization in sparsely populated regions has resulted in a continued decrease in the number of small high-schools.

All States provide public schools and permit students between the ages of 6 and 20 years to attend. Most States have enacted compulsory attendance laws for certain age-groups. The compulsory attendance ages range from 6 to 18 years, but a majority of the States require attendance between the ages of 7 and 17 years. Of the total population of persons between 6 and 17 years old in October 1957, 96·5 per cent were enrolled in school. At that time 93 per cent of all school-age children, generally defined as those between 5 and 17 years old, inclusive, were enrolled. In elementary and secondary schools the proportion of boys and girls was about the same but in higher-education institutions men made up about two-thirds of the student body and women one-third.

School enrolments increased for the thirteenth consecutive year. In 1957–58, 43,135,000 persons, or about one out of every four in the population, were attending public or private schools and colleges; an increase of more than 4 per cent over 1956–57.

The latest available data indicate that about one-half of the high-school graduates now go to college, about 42 per cent full time and 8 per cent part time. Attendance of students at institutions of higher education is altogether voluntary. Assuming that they meet admission requirements, students are free to choose the type of institution they attend—public or private, liberal arts or technical, 2-year or 4-year, and to pursue any curriculum or prepare for any profession to the extent of their abilities. A student in an institution may of his own volition drop out altogether or transfer to another institution. In the autumn of 1957, colleges and universities enrolled more than 3 million students, an increase of 4 per cent over the autumn 1956 enrolment, and a 43 per cent increase over the 1952 autumn enrolment. The number of freshmen entering college in the autumn of 1957 represented slightly more than 30 per cent of persons in the country who were 17 years of age in 1956. About 58 per cent of the students enrolled were in public institutions. Enrolment in public institutions is increasing more rapidly than in private. About 800,000 of the students attending full time lived in dormitories provided by the institutions.

The degree-granting colleges conferred a total of 411,000 degrees in 1957–58, 8·3 per cent more than in 1956–57. Of the total conferred, 82·8 per cent were bachelor's, 15·1 were master's and 2·1 were doctor's degrees. The average cost of a year in college was between 1,500 and 2,000 dollars, and the median award in scholarship aid was less than 300 dollars.

In 1957–58 between 30 and 35 million adults participated in adult education programmes sponsored by industry, labour unions, the Armed Services, farm organizations, and other groups. In carrying out their programme, these groups had the co-operation of public libraries, public school systems, higher institutions, television systems and Government agencies.

Extensive research is carried on by public and private agencies such as colleges and universities, State departments of education, and various philanthropic groups. Their research is directed toward solving some of the problems facing education; for example, it includes further investigation of the learning process and the character and extent of individual differences. State departments and local school systems direct their research primarily to solving local problems, and colleges and universities direct theirs to broader problems in education.

## THE PHYSICAL SOCIETY, 1958—59

THE annual general meeting of the Physical Society was held on May 21 at the Royal Institution, London, and immediately following the meeting Mr. J. A. Ratcliffe delivered his presidential address entitled "Recent Trends in the Theory of the Ionosphere". The report of the council of the Society and the accounts and balance sheet for 1958 were adopted at the meeting and the composition of the new council to hold office for the session 1959–60 was announced.

The income of the Society during 1958 exceeded expenditure by £4,581 and was mainly due to the increase in price of the Society's publications when sold to the general public which the council authorized in 1957. Notwithstanding the rise in price, sales have increased. The membership rose from 2,069 to 2,136, but the increase was entirely in the student membership grade. The forty-second annual exhibition of scientific instruments and apparatus was held during March 24–27 in the two halls of the Royal Horticultural Society. The size of the exhibition and attendance were similar to those of the previous year. The sales of the exhibition handbook and the receipts from exhibitors resulted in a satisfactory surplus of £5,905, of which £4,000 was transferred to the exhibition contingency fund and the remainder to the general income and expenditure account.

The council's report refers briefly to the activities of the Society during the year and in particular to the conferences of two or three days duration which were held on various subjects in Cambridge, Durham, Malvern and Swansea. The attendances were usually between 200 and 250, of which approximately half on the average were members of the Society. A few research students and others were financially assisted to attend these conferences by means of a grant allotted to the Society by the Royal Society. The decision to recombine the two sections of the Society's *Proceedings* was put into effect during 1958 and the volume of work published (208 original articles, 74 research notes and 21 letters to the editor) was substantially the same as in 1957. Vol. 21 of the "Reports on Progress in Physics", which was published during the summer, contained nine articles, and these articles were also available for purchase separately.

The informal discussions with the Institute of Physics which were begun in 1957 to consider the

closer co-operation between the Institute and the Society were continued. A mutually agreed document entitled "Memorandum to Members—Proposal to Amalgamate the Institute of Physics and the Physical Society" was circulated, together with an explanatory letter from the president. A joint amalgamation committee has been set up and is now engaged in more detailed discussions.

At the annual meeting, the president, Mr. J. A. Ratcliffe; the honorary secretaries, Dr. C. G. Wynne, Dr. H. H. Hopkins and Mr. A. G. Peacock; the honorary foreign secretary, Prof. E. N. da C. Andrade; and honorary treasurer, Dr. D. A. Wright, were re-elected to serve for 1959–60. The newly elected vice-presidents were: Prof. F. Llewellyn Jones and Dr. G. B. B. M. Sutherland, and the newly elected members of council: Mr. D. W. Fry, Dr. V. E. Cosslett, Prof. F. C. Frank, Prof. W. E. Burcham, Dr. R. L. F. Boyd, Dr. R. A. Smith and Prof. D. H. Wilkinson.

S. Weintroub

## SCHOOL MEALS IN ASIA AND THE FAR EAST

VARIOUS Food and Agriculture Organization conferences, as well as regional nutrition meetings convened periodically in co-operation with the World Health Organization, have emphasized the importance of supplementary feeding as a means of improving the nutrition of vulnerable groups. The First Regional Nutrition Committee in South and East Asia, which met in Baguio, the Philippines, in 1948, recommended a type of meal which could be supplied to school-children in the region. This meal pattern emphasized the use of cheap, locally available foods that would provide the children with all essential nutrients.

The Fourth Regional Nutrition Committee of the two Organizations, which met in Tokyo in 1956, considered a number of important factors relating to school feeding programmes; it recommended that the Food and Agriculture Organization should convene a school feeding seminar for countries in South and East Asia, at which the future development of school-feeding along sound lines could be discussed by appropriate country representatives.

Much of the Food and Agriculture Organization's practical work in school-feeding has been done in co-operation with the United Nations Children's Fund, the Organization providing the technical guidance in organizing and developing programmes based initially on dried skim milk and other supplies made available by the Fund. This Fund has become increasingly interested in the long-term development of measures to improve the nutrition of children and has recently been authorized to increase the scope of assistance which it can provide. It was agreed, therefore, that the Fund should join the Organization in convening the seminar. Because malnutrition is often a serious problem among children of pre-school age, it was also agreed that consideration would be given to this important group of the population.

The seminar was designed to bring together, from the countries concerned, workers associated with various aspects of child-feeding programmes, in particular school-feeding programmes, for consideration of the problems met in developing them and of measures needed to improve and expand them on a sound nutritional and financial basis. The Government of Japan extended an invitation for the seminar to be held in Japan, and it was held in International House, Tokyo, during November 10–19, 1958. The seminar was attended by delegates from twelve countries in the region, as well as by representatives from the World Health Organization International Co-operation Administration, and Co-operative for American Remittances to Everywhere. A report on the seminar has now been issued (H.M.S.O., 2*s*. 6*d*.).

## INDUSTRIAL HEALTH IN THE POTTERIES

DURING 1956–58, four members of the factory inspectorate carried out a survey of industrial health in the pottery industry in the Stoke-on-Trent area. The survey was undertaken with the advice of the Industrial Health Advisory Committee. This Committee was set up in 1955 by the Minister of Labour and National Service to advise him on measures to further the development of industrial health services in work-places covered by the Factories Acts.

On the advice of the Committee the Minister instituted two industrial health surveys, which were to be regarded as pilot surveys. The first was of all the factories in a particular area—the town of Halifax was chosen—and the report on that survey was published in 1958. The second was a survey of a specific industry—the pottery industry.

A number of considerations led to the choice of the pottery industry. Among them was the fact that it is geographically compact, and that, although over a number of years much has been done in the industry to eliminate or reduce the known health risk, it was considered that a survey of the pottery industry would have particular interest in giving an opportunity to assess both the success of the measures so far taken and the continuing needs.

Although a survey of this kind offers no basis for comparing conditions in the pottery industry with those of other industries, it is possible to draw some comparison between present conditions in the pottery industry and those of the past. General conditions in the industry to-day are markedly different from what they were. The industry has done a great deal, particularly in the years since the end of the Second World War, to improve working conditions and to reduce the health hazards connected with pottery manufacture.

The classic industrial disease of the pottery industry was lead poisoning, due in part to the lead glazes used. By the middle 1940's the use of low-solubility

or leadless glazes had become so widespread that it was considered practicable to prohibit the use of any glaze that was not either leadless or of low solubility. This was not an easy requirement for all firms to comply with, and the glazed tile industry was faced with a particularly difficult problem. Intensive research enabled all firms to be in a position to comply with the requirement when it became law. The other source of lead poisoning was the colour used in decorating the ware. Higher standards of cleanliness and improved methods of dust control have, so far, dealt with this hazard, with the satisfactory result that in recent years lead poisoning has virtually been eliminated from the industry.

Another major achievement of the industry, this time in reducing the risk of pneumoconiosis, has been the substitution of alumina for powdered flint in the placing of china for the biscuit fire. When it became clear that alumina was a satisfactory alternative, the china industry, in spite of some technical problems involved, agreed that the flint should be replaced by alumina. By 1947, when this change was made compulsory, all firms in the industry had in fact changed over.

Work on the control of dust in the making processes, where there is a health hazard from pneumoconiosis, is continuing. For some years the British Ceramic Research Association has been working on the dust problems of the industry, and has done much valuable research work into the behaviour and control of dust given off in certain processes. The Research Association has already designed dust-control plant for the processes of towing and hollow-ware fettling which is proving most effective. Work is in progress on the control of dust in the dust tile-making processes, and to determine the most suitable material for workers' overalls where there is need to protect them against dust.

To provide a continuing forum for discussion of the health and safety problems of the industry, the Chief Inspector of Factories in 1956 appointed a Joint Standing Committee of the Pottery Industry. With the help of the British Ceramic Research Association, it has published an advisory booklet on dust-extraction in the pottery industry. It has also directed the attention of industry to the dangers inherent in the use of hydrofluoric acid in cleaning gold, encouraging the use of other methods which it has made known.

The aim of the survey was to present an objective picture of existing conditions in the industry and to indicate outstanding problems. The visits made by the inspectors have been followed up by action to secure improvements. The work that requires to be done in order to deal with outstanding problems is under examination by the Joint Standing Committee (H.M.S.O., 5*s*.).

## DEVELOPMENTS IN TRAINING

A SERIES of five papers on training were given at the Polytechnic, Regent Street, to an audience of two hundred directors and industrial executives during January and February, 1957. They have now been reprinted and form a valuable addition to the scanty information available for those concerned with all aspects of training in industry*.

The first, by Prof. J. Z. Young, deals with the fundamental aspects of learning by drawing on biological studies of organisms at all levels of complexity. Developing the importance of perception in learning, W. D. Seymour, of the Department of Engineering Production in the University of Birmingham, produces evidence to show that carefully devised training procedures for manual skills which take account of recent findings will usually halve the normal learning period. In the third lecture, Mrs. W. Raphael, assistant director of the National Institute of Industrial Psychology, describes the in-plant training being carried out in seven European countries and shows that the training provided for operatives exists largely in name only. F. A. Heller, head of the Department of Management Studies at the Polytechnic, shows how the development of managerial skills can be approached from the same biological and analytical points of view as any other skill training. Like other lectures in the series, he pays special attention to the methods rather than to the content of training programmes. The fifth lecture was given by S. D. M. King, director of Organization and Training, Ltd., who used case-studies to illustrate the importance of relating training to a carefully devised policy at all levels of an organization.

* New Developments in Training: Five Studies in the Efficient Communication of Skills. Edited by Frank A. Heller. (New Development Series No. 3.) Pp. 80. (London: Polytechnic Management Association, 1959.) 5*s*.

## ERGONOMICS

THE development of modern industry with the substitution of mechanization for craftsmanship has brought new problems; the machine has reached the point where it is no longer the limiting factor in production and this in turn is imposing new stresses and strains on the operator, who can no longer be left to get along as best he can. That this was beginning to happen began to be realized about fifty years ago, and early developments in fitting the job to the man, like the motion study of Gilbreth, were part of scientific management for increasing production through reduction of fatigue. Men of the biological sciences began to become aware that people at work were worthy of study and that remarkably little was known about their capabilities and aspirations.

It took the added stress of two world wars to stimulate any real interest. A start was made in the First World War when groups of physiologists and psychologists such as the Industrial Fatigue Research Board in the United Kingdom started work. Between the wars progress was slow, perhaps because a general

labour surplus removed a demand for maximum economy in use of labour; but during and since the Second World War the research effort has been vastly intensified. Much of it gained its initial impetus from the armed forces and has, particularly in the United States, continued to be supported by them. In other countries research has been much more directed towards solving industrial problems, as typified by that done by work physiologists in Sweden and Germany.

The fragmentation of the subject into a number of disciplines independently studying human work could not continue indefinitely, and the first fusion occurred in the United Kingdom in 1949 with the formation of an interdisciplinary society and the coining of the term 'ergonomics' to cover the study of human work. Although the British society attracted adherents from all over the world it soon became clear that a truly international meeting was needed. The initiative was taken by the European Productivity Agency in the form of Project 335, the final aim of which is a tripartite international conference of scientists, employers and employees. As a preparation for this, two preliminary steps were taken. The first was to send a mission to the United States to report on the situation there, and the second was to hold a technical seminar to assemble information on progress in Europe. A report has now been issued, part one of which contains the report of the mission and part two a report on the seminar*. The tripartite conference has still to take place.

* "Fitting the Job to the Worker. A Survey of American and European Working Conditions in Industry" (Paris, O.E.E.C., 1958).

# AN OCEANOGRAPHIC SURVEY OF THE ROSS SEA

By J. S. BULLIVANT

New Zealand Oceanographic Institute, Department of Scientific and Industrial Research, Wellington, New Zealand

IN January 1959 an oceanographic survey of the Ross Sea was carried out by members of the New Zealand Oceanographic Institute, from the New Zealand Antarctic Supply Ship H.M.N.Z.S. *Endeavour*.

The route taken by the *Endeavour* and the distribution of the twenty-four stations occupied are shown in Fig. 1. A brief station list is also included, particularly for the information of workers planning investigations in the area in the near future.

The aim of the survey was to investigate the hydrology, the benthic fauna and the marine sediments in the area.

The routine procedure at each station was to lower a bathythermograph (275 m.) and a cast of reversing bottles, make a vertical plankton haul from near the bottom to the top, collect phytoplankton, make three lowerings with a twin orange-peel grab sampler and collect epifauna from the bottom with a trawl. The grab sampler consisted of two modified four-blade orange-peel grabs, each having a bucket capacity of 24·5 litres, suspended one at each end of a 4-ft. bar.

In addition to these routine observations, surface water samples from stations *A*466 and *A*470 and a bottom water sample from *A*470 were obtained for determination of carbon-14 activity in order to study water movements in the Ross Sea and under the Ross Ice Shelf.

An underwater-camera and a bottom trawl were used at three stations (*A*468, *A*469 and *A*471) near Ross Island, to sample three different types of bottom community. Some of the photographs

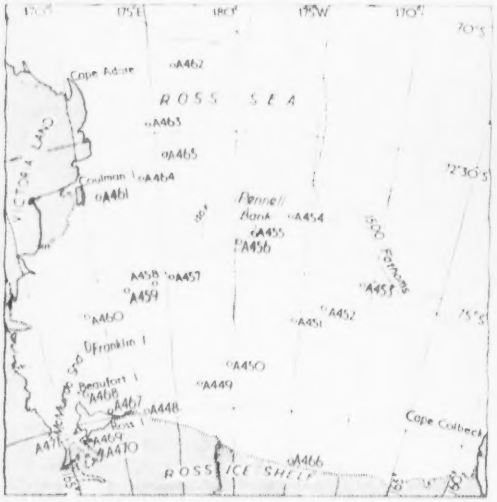



Fig. 1. Ross Sea, Antarctica, showing stations occupied from H.M.N.Z.S. *Endeavour*

Fig. 2. Bottom photograph from station *A*468; sponges, coelenterates and bryozoans predominate. Note crinoid top left. (Depth, 110 m.; foreground, 0·75 m. wide; depth of field, 1 m.)

Table 1. List of Stations Occupied from H.M.N.Z.S. *Endeavour* in the Ross Sea

| N.Z.O.I. station No. | Position | Date | Provisional depth (m.) | Nature of bottom | Serial hydrological observations to: (m.) | Trawls | Grabs | Phyto-plankton | Plankton | Other |
|---|---|---|---|---|---|---|---|---|---|---|
| 1448 | 77° 27′ S., 172° 22′ E. | 10.1.59 | 752 | mud | 705 | × | × | × | × | |
| 1449 | 77° 05′ S., 177° 12′ E. | 11.1.59 | 362 | mud | 265 | × | × | × | × | |
| 1450 | 76° 42′ S., 179° 44′ E. | 11.1.59 | 472–318 | muddy sand | 265 | × | × | × | × | |
| 1451 | 76° 00′ S., 175° 25′ W. | 12.1.59 | 523 | gritty mud | 515 | × | × | × | × | |
| 1452 | 75° 35′ S., 173° 18′ W. | 12.1.59 | 1,280 | yellow-brown mud | 1,300 | — | × | × | × | |
| 1453 | 75° 09′ S., 171° 00′ W. | 13.1.59 | 2,195 | yellow-brown mud | 1,400 | — | × | — | × | |
| 1454 | 73° 56′ S., 176° 30′ W. | 14.1.59 | 914–828 | rocks | 255 | × | — | × | × | |
| 1455 | 74° 22′ S., 178° 35′ W. | 15.1.59 | 322–340 | stones, muddy sand | 320 | × | × | — | × | |
| 1456 | 74° 30′ S., 179° 40′ W. | 15.1.59 | 238–201 | stones, gritty mud | 180 | × | × | — | × | fishing line |
| 1457 | 75° 02′ S., 175° 50′ E. | 16.1.59 | 315–342 | mud | 312 | × | × | × | × | |
| 1458 | 75° 10′ S., 174° 00′ E. | 16.1.59 | 461–486 | muddy sand | 436 | × | × | × | × | corer |
| 1459 | 75° 17′ S., 172° 20′ E. | 16.1.59 | 534–549 | soft mud | 510 | × | × | × | × | corer |
| 1460 | 75° 38′ S., 168° 32′ E. | 17.1.59 | 415–430 | gritty mud | 395 | × | × | × | × | corer |
| 1461 | 73° 32′ S., 171° 22′ E. | 18.1.59 | 578–567 | sandy mud | 555 | × | × | × | × | corer |
| 1462 | 71° 15′ S., 176° 30′ E. | 20.1.59 | 1,831–2,381 | gritty mud | 1,750 | — | × | × | × | |
| 1463 | 72° 20′ S., 174° 50′ E. | 21.1.59 | 468–465 | barnacle plates | 435 | × | × | × | × | |
| 1464 | 73° 20′ S., 174° 00′ E. | 22.1.59 | 369–384 | sand pebbles | 345 | × | × | × | — | |
| 1465 | 72° 55′ S., 175° 30′ E. | 22.1.59 | 399 | barnacle plates | 375 | × | × | × | — | |
| 1466 | 78° 26′ S., 174° 50′ W. | 24.1.59 | 569 | mud | 570 | × | × | × | — | corer 9C sample |
| 1467 | 77° 25′ S., 169° 28′ E. | 26.1.59 | 88–183 | rocks | — | × | — | — | — | camera |
| 1468 | 76° 59′ S., 167° 36′ E. | 26.1.59 | 110 | — | — | × | — | — | — | camera |
| 1469 | 77° 50′ S., 166° 33′ E. | 29.1.59 | 64 | gritty mud, spicules | — | — | × | × | × | camera |
| 1470 | 77° 50′ S., 166° 30′ E. | 4.2.59 | 377 | muddy sand | 365 | — | × | — | × | 9C samples |
| 1471 | 77° 37′ S., 166° 20′ E. | 6.2.59 | 165–69 | — | — | × | — | — | — | camera |

revealed a surprisingly dense epifauna, dominated by sponges and Bryozoa (Fig. 2).

A gravity corer was used at stations *A458*, *A459*, *A460*, *A461* and *A466*; short cores were secured.

Hourly surface water samples were taken on passage out of McMurdo Sound, and continuous surface temperatures were recorded during the greater part of the cruise.

Of particular interest was the discovery of large deposits of calcareous barnacle plates. The plates were found sparsely distributed over the whole of the Ross Sea; but at stations *A463* and *A465* in approximately 460 m. and 400 m., respectively, these plates, together with scattered rocks on which a live barnacle was growing, formed the bulk of the bottom deposit. The deposit also contained a small percentage of the calcareous remains of molluscs, bryozoans and corals, but as far as penetrated by the grabs and trawl was entirely free of mud or sand. The living and dead barnacles belong to the genus *Hexelasma*. Estimates of the age of the barnacle plates are to be made from determinations of their carbon-14 activity.

The biological material collected is a substantial addition to that already available from Antarctic waters.

Results of the survey will be published by the New Zealand Department of Scientific and Industrial Research.

The wholehearted support the expedition received from the commanding officer and ship's company of H.M.N.Z.S. *Endeavour* is gratefully acknowledged.

# CHANGE OF COSMIC RAYS IN SPACE

By Prof. H. V. NEHER

Norman Bridge Laboratory of Physics, California Institute of Technology, Pasadena

THE advent of space rockets now makes possible direct measurements on quantities that have heretofore suffered from the interference of the Earth, its atmosphere or its magnetic field. Among these are measurements on cosmic rays. The absorption of the radiation in the Earth's atmosphere and the analysing effect of its magnetic field have, however, yielded valuable information on the total energy content and the individual particle energy. From such measurements, together with a knowledge of their chemical composition, one can deduce the intensity or number of primaries in space. When this is done, using results collected over a period of years obtained with balloons at various latitudes, one finds surprisingly large time variations in the numbers of primary particles. In what follows we discuss what such measurements have so far disclosed about the absolute intensity and how it varies with time. Some preliminary measurements have already been made in rockets, and further measurements, giving more complete checks on these calculations, will undoubtedly come in the future.

A brief note concerning these large changes, based on data taken in 1954 and 1957, has already been published[1]. We wish here to present two additional methods of arriving at the numbers of primary particles in 1954 together with new results taken near the north geomagnetic pole in the summer of 1958 when the intensity there was even lower than in 1957.

The period 1954–58, when solar activity went from a minimum to a maximum of activity, provided

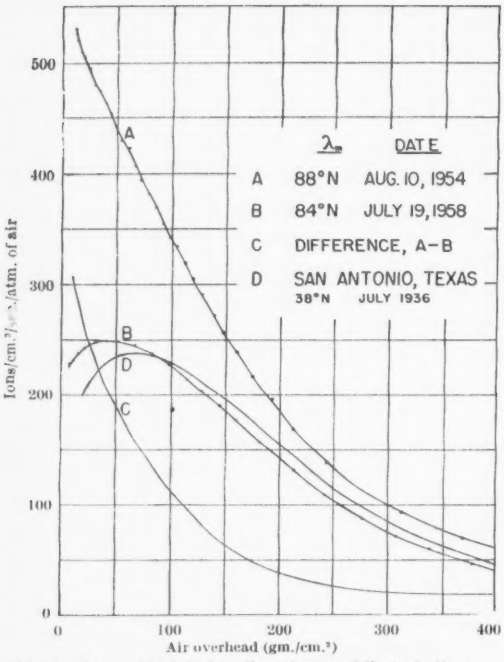



Fig. 1. Curves *A* and *B* show the extremes of the ionization so far measured with balloons near the north geomagnetic pole. Curve *A* represents data taken at the solar minimum of 1954, while curve *B* represents the situation near the solar maximum of 1958. The absorption in the atmosphere of the particles present in 1954 but which were absent in 1958 is given by curve *C*

an excellent opportunity to study the changes produced in cosmic rays. Fig. 1 shows how large these changes have been near the north geomagnetic pole. The behaviour of curve *A* was typical of the curves from five similar balloon flights[2] made with ionization chambers in July and August 1954. All five records showed the same turn-up of the ionization curve at about 15 gm. cm.$^{-2}$. It was shown in ref. 2 that the behaviour of curve *A* at low pressures was consistent with the absorption of low-energy particles and that the turn-up was probably due to the absorption of protons with energies down to at least 150 MeV.

By 1958, the character of the radiation at the pole was radically different. The largest decrease occurred in the year 1956–57[1]. As shown by curve *B*, the ionization at high altitudes was less than half its value in 1954. In fact, the area under curve *B* is about 3 per cent less than the area under the curves taken in 1936 at San Antonio, Texas[3] (geomag. lat. 38° N.). It should be remarked that we feel confident that a direct comparison can be made between these years, for we not only have instruments which we have compared through the years but we have also checked their absolute calibration by using standard capacitors.

Our first method of estimating the number of primaries in 1954 is to calculate the number causing the difference between 1954 and 1958 and then adding to this the number present in 1958. To arrive at this latter figure we note that due to the nearly equal areas under curves *B* and *D* of Fig. 1 and to the similarity of shape, the number of primaries must be about the same. Taking the difference in area at the lower pressures and assuming a mean energy of 3 BeV. per particle causing this difference, we find that the number of primaries involved is approximately 0·007 cm.$^{-2}$ sec.$^{-1}$ sterad.$^{-1}$. Previous calculations[4] have shown that the number of primaries present at San Antonio in 1936 was 0·040 cm.$^{-2}$ sec.$^{-1}$ sterad.$^{-1}$. This number is consistent with the measurements of MacDonald[5] at the equator, together with the increase in energy brought in by the primaries as one goes from the equator to 38° N. We thus find that the number of primaries present in 1958 near the pole was 0·047 cm.$^{-2}$ sec.$^{-1}$ sterad.$^{-1}$.

Referring now to Fig. 1, curve *C* shows the difference between *A* and *B* and represents the absorption in the atmosphere of those particles that were present in 1954 but were absent in 1958. A Gross transformation of this difference curve shows that the major part of the area under the curve is due to particles the effect of which varies nearly linearly with thickness of atmosphere. The fact that curve *C* has a tail that extends even to sea-level is undoubtedly due to mesons formed in the upper part of the atmosphere and especially by the higher-energy primaries that also change during a solar cycle.

Ignoring the high- and low-pressure ends, the bulk of the area may be accounted for by particles with a maximum range of about 300 gm. cm.$^{-2}$ in air. Assuming a range proportional to the energy, we may say with sufficient accuracy that the Gross transformed curve corresponds to a differential number distribution that is independent of energy.

We shall here assume that 15 per cent of the primaries responsible for curve *C* are α-particles, 2 per cent of average $Z = 8$ and the remainder protons. We find that we need protons of mean energy 0·55 BeV., α-particles of mean energy 2·2 BeV. and for $Z = 8$, a mean energy of 1·2 BeV. per nucleon, for each to have a mean range of 150 gm. cm.$^{-2}$. We then arrive at a weighted mean energy of 1·5 BeV. per particle. The area under curve *C* is $1{\cdot}00 \times 10^9$ eV. cm.$^{-2}$ sec.$^{-1}$. Hence the number of particles per unit of horizontal area is 0·67 cm.$^{-2}$ sec.$^{-1}$. Thus per unit of solid angle we have, 0·21 cm.$^{-2}$ sec.$^{-1}$ sterad.$^{-1}$.

To arrive at the total number of primaries at the pole in 1954 we add to the above the number present in 1958. We have already found this to be 0·047 cm.$^{-2}$ sec.$^{-1}$ sterad.$^{-1}$. In this manner we find the total at the top of the atmosphere near the pole in 1954 to be 0·26 par. sec.$^{-2}$ sec.$^{-1}$ sterad.$^{-1}$.

Another method of arriving at the number of particles responsible for the difference between 1954 and 1958 is to estimate the mean specific ionization per particle at the top of the atmosphere and, knowing the ionization, the number of particles immediately follows.

Taking the mean energies of the protons, α-particles and average $Z = 8$ for the heavy primaries, as given above, we find that the specific ionization relative to that for a minimum ionizing particle of unit charge has the values 1·3, 5·2 and 64 respectively. Using the relative abundances cited before and published curves on energy loss, we find the average specific ionization for these time-varying particles at the top of the atmosphere at the pole to be 210 ion pairs cm.$^{-1}$ in air at 1 atm. If $\bar{\sigma}$ is this mean specific ionization and there are $J$ particles cm.$^{-2}$ sec.$^{-1}$, then the ionization, $I = \bar{\sigma}J$. Since $I = 350$ ions cm.$^{-2}$ sec.$^{-1}$ atm.$^{-1}$ of air from Fig. 1, then $J = 1{\cdot}67$. The instrument is receiving particles from a solid angle of $2\pi$. Hence the uni-directional intensity is 0·26 par. cm.$^{-2}$ sec.$^{-1}$ sterad.$^{-1}$. This method then gives a

total of 0·31 par cm.$^{-2}$ sec.$^{-1}$ sterad.$^{-1}$ at the pole in 1954.

A third method of arriving at the numbers of particles is to take the increments in the area under the ionization-depth curves for changes of latitude. This was done in 1954 from Boston to Thule, Greenland, using Bismarck as a base station to take account of temporal changes. The results of this analysis have been published[3]. These calculations gave a total of 0·24 par. cm.$^{-2}$ sec.$^{-1}$ sterad.$^{-1}$ at the pole.

These three values, 0·26, 0·24 and 0·31, then give an average of 0·27 par. cm.$^{-2}$ sec.$^{-1}$ sterad.$^{-1}$ at or near the north geomagnetic pole in the summer of 1954. An application of Liouville's theorem tells us that with an isotropic distribution at infinity, this was also the intensity in space at that time. The corresponding total intensity in space was then 3·1 cm.$^{-2}$ sec.$^{-1}$ through a sphere of unit area.

In 1958, the intensity at or near the pole was 0·047 cm.$^{-2}$ sec.$^{-1}$ sterad.$^{-1}$ or 0·59 cm.$^{-2}$ sec.$^{-1}$, as discussed above. We thus arrive at the conclusion that the numbers of particles in space near the Earth, but sufficiently far removed to be free from any of its effects, changed by a factor of 5 during this period of four years. This is probably a lower limit, for we do not know how high the ionization would have gone in 1954 had our balloons gone higher.

So far, data on the number of cosmic-ray particles in space have been obtained by both the United States and the U.S.S.R. On the journey of *Pioneer IV* which went near the Moon and is presumably now in orbit around the Sun, the data at large distances gave 1·8 ± 0·3 particles cm.$^{-2}$ sec.$^{-1}$. (I am grateful to Prof. J. A. Van Allen for giving me this figure.) This rocket was launched on March 3, 1959. For the Russian cosmic rocket, launched on January 2, 1959, the value measured[6] was 2·3 cm.$^{-2}$ sec.$^{-1}$. These values are to be compared with the above calculated values using ionization chambers of 0·59 cm.$^{-2}$ sec.$^{-1}$ in 1958 and 3·1 cm.$^{-2}$ sec.$^{-1}$ in 1954.

There are at least two reasons why the values measured in these rockets at the beginning of 1959 are higher than those calculated from terrestrial measurements in the summer of 1958. (1) Cosmic-ray intensity near the orbit of the Earth appears to have reached a minimum near the summer of 1958 and had definitely started to climb[7] by January of 1959. (2) The instruments in each of the rockets were surrounded by the material of the vehicle. One would therefore expect the number of secondary particles to be an important factor. This would be especially true for the Soviet rocket, which was quite massive. To avoid the uncertainty of the contribution of surrounding matter, the detecting instrument would need to be ejected from the vehicle and remain at some distance away.

For those interested in space travel, an estimate may be made of the radiological intensity of cosmic rays in space. From Fig. 1 we see that the ionization at the pole was at least 530 ions cm.$^{-2}$ sec.$^{-1}$ atm.$^{-1}$ of air at the highest altitude reached. In space, where the shielding effect of the Earth is missing, this number would be just doubled. In terms of radiological units we would then expect at a solar minimum to have at least 1,060 ions cm.$^{-3}$ sec.$^{-1}$ atm.$^{-1}$ of air = 1·8 mr. (milliroentgens) per hr. At the solar maximum of 1958, we found the total intensity in space across unit sphere to be 0·59 par. cm.$^{-2}$ sec.$^{-1}$. Assuming an average specific ionization of 200 ions cm.$^{-1}$ of path in air at 1 atm., we find an ionization of 120 ions cm.$^{-3}$ sec.$^{-1}$ atm.$^{-1}$ of air or 0·21 mr. per hr.

It is to be hoped that as time progresses data from instruments in rockets will give us more definite information as to the mechanism that causes these large changes in the primary cosmic radiation.

The assistance of the Office of Naval Research in making the necessary arrangements to carry out this programme is greatly appreciated. I would also like to thank the Office of Naval Research, the Atomic Energy Commission and the National Academy of Sciences through the International Geophysical Year for financial support.

[1] Neher, H. V., and Anderson, Hugh, *Phys. Rev.*, **109**, 608 (1958).
[2] Neher, H. V., *Phys. Rev.*, **103**, 228 (1956).
[3] Millikan, Neher and Haynes, *Phys. Rev.*, **50**, 992 (1936).
[4] Neher, H. V., *Phys. Rev.*, **83**, 649 (1951). See also Neher, H. V., "Annual Review of Nuclear Science", **8**, 217 (1958).
[5] MacDonald, F. B., *Phys. Rev.*, **109**, 1367 (1958).
[6] Vernov, S. N., Chudakov, A. Ye., Vakulov, P. V., and Logachev, Yu. I, Moscow, *Doklady Akademii Nauk SSSR.*, **125**, 304 (1959).
[7] Results to be published.

# STUDIES OF COAL

## By STAFF OF THE BRITISH COAL UTILISATION RESEARCH ASSOCIATION

### Carbonization of Coals in the Presence of Activated Charcoal

IT has been reported recently by Adams *et al.*[1] that the pyrolysis products of coal, if left in contact with a hot carbonaceous surface, undergo further reactions. Experiments in our laboratories[2] have yielded further results of a similar kind with respect to the behaviour on heating of coal mixed with, or overlaid by, charcoal.

Measurements of the amount of tar-like pyrolysis products emitted on burning briquettes made from mixtures of a low-rank coal and an activated charcoal (prepared from a coal char) showed a decrease as the proportion of coal in the mixture decreased, but more markedly than was to be expected from a dilution effect. On the other hand, briquettes made from the same coal mixed with an unactivated charcoal did not show a disproportionate reduction. It thus appears that the tar-like pyrolysis products evolved during heating were cracked on the extensive surface of the activated charcoal. The surface area that would be accessible to the large molecules likely to be present in these volatile vapours would be about 200 m.$^2$/gm., whereas the accessible surface area of the unactivated char would be less than 5 m.$^2$/gm.

When mixtures of coal and activated charcoal, or beds of coal overlaid with activated charcoal, were heated in a slow stream of nitrogen, or at a reduced pressure, to about 600° C., no tarry material was formed; instead, it was possible to collect in cooled traps an almost colourless liquid. This liquid was found, by infra-red analysis, to be composed of simple organic molecules (the benzene and toluene yield was in the range 0·3–0·7 per cent of the dry coal weight, compared with 0·1 per cent from the coal alone).

Table 1. Yields of Products from the Carbonization to 600° C. of a Low-rank Coal (N.C.B. Type 902) overlaid with Activated Charcoal
(Percentages of dry coal weight)

| Ratio of charcoal to coal | Acidic aqueous liquor | Tar oil | | Combustible gas | | Carbon dioxide | Carbon deposited in the charcoal | Total |
|---|---|---|---|---|---|---|---|---|
| | | Total | Benzene + toluene | Total | Paraffin to hydrogen ratio | | | |
| 0 | 9·7 | 10·0 | 0·11 | 7·2 | 1·5 | 3·0 | 0 | 29·9 |
| ¼ | 9·7 | 2·7 | 0·36 | 8·8 | 1·5 | 3·7 | 5·3 | 30·2 |
| ½ | 9·7 | 1·4 | 0·46 | 8·5 | 1·4 | 4·7 | 5·5 | 29·7 |
| 1 | 9·7 | 1·2 | 0·72 | 8·5 | 1·3 | 6·8 | 6·0 | 31·2 |

The gaseous material evolved from the bed was found to be different from that obtained from the carbonization of the coal alone and, further, it was deduced from weight balances for the system and from analysis of the charcoal after use that carbon had been deposited in the charcoal.

In Table 1 are compared the results obtained from the carbonization to 600° C. of a low-rank coal (N.C.B. type 902) with those from the same coal overlaid with an activated charcoal.

When smaller proportions of charcoal were used in the carbonization experiments it was found that the condensates became slightly coloured and their complexity was increased. The charcoal could be used again, provided the deposited carbon from the cracking process, which reduced the effectiveness of the charcoal, was removed by oxidation, for example, with steam at about 900° C. There seemed to be little doubt that to achieve the complete elimination of dark tarry material from the condensates it was necessary for the vapours of the pyrolysis products of the coal to encounter a substantial amount of carbon surface. The effect reported by Adams *et al.* is not as great as that which we have observed ; the reduction by only 1 per cent of the amount of tarry matter formed, and the slight increase in the amount of liquor, suggest that but little carbonaceous surface was available for cracking the coal 'volatiles'.

It could be concluded that if a large amount of a pre-carbonized char, sufficient to have the necessary available surface area, was overlaid upon a 'green' coal and the whole was heated, very little tar would be formed, although some benzole would be obtained together with a gas of useful calorific value.

R. L. Bond
A. M. Godridge
A. R. Murnaghan
D. H. Napier
D. J. Williams

[1] *Nature*, **183**, 33 (1959).
[2] Brit. Coal Utilisation Res. Assoc. Ann. Rep., 38 (1956).

## Smoke Emission from Coal and Low-Temperature Chars

Work by this Association in a laboratory apparatus[1-3] has confirmed the observations of Piersol[4], afterwards verified by Adams, Gaines, Gregory and Pitt[5], that volatile matter is not an adequate guide for the amount of smoke produced from chars. In Fig. 1 it will be seen in all cases that chars prepared from a low-rank coal produce less smoke than coals with equivalent volatile matter. Although Piersol[4] claimed a straight-line relation between the amount of smoke liberated under standard conditions of testing and the percentage of volatile matter in a range of coals, we did not find a direct proportionality (Fig. 1). There is a general tendency for smoke emission to decrease with decreasing volatile matter, but highly caking and swelling coals tend to mask this trend. McHugo, Shaw and Whittaker[6], who burnt a range of coals in a domestic appliance, observed a similar effect.

We found a relation between tar-yields (Gray King assay at 600° C.) and smoke emission for a number of samples, similar to that found by the Coal Research Establishment workers[6]. We decided to extend the investigation into the relation between the hydrogen content of coals and their smoke emission for the following reasons.

(1) Spooner[7] maintained that the tar yield of bright coals was related to their hydrogen contents. Bradbury and Mott[8] therefore stated that, since tar yield and smoke emission are related, either the hydrogen content or the tar yield should serve as a guide to the amount of smoke produced. We have also established a definite correlation between the tar yields (not given in this communication), and the hydrogen contents for the fuels which we examined (eighteen), the correlation coefficient being calculated as 0·93, which is found to be significant at the 0·001 level of probability.

(2) Smoke is probably related in some manner to the chemical constitution of the fuel, particularly to the size and stability of the condensed aromatic ring clusters and the number of edge groups, which in turn are related to hydrogen content.

(3) We have shown by statistical examination that when weight of smoke is plotted against volatile matter (Fig. 1) the high-swelling coals follow a different relation from that for the low-swelling coals, the high-swelling coals producing more smoke than low-swelling coals with equivalent volatile matter.

Fig. 2 shows the general relation between the weights of smoke and the hydrogen contents of the coals and chars tested. Both high- and low-swelling coals appear to follow the same relation.

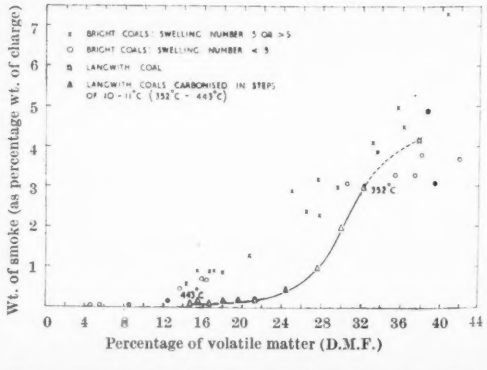

x BRIGHT COALS: SWELLING NUMBER 5 OR >5
○ BRIGHT COALS: SWELLING NUMBER < 5
B LANGWITH COAL
A LANGWITH COALS CARBONISED IN STEPS OF 10 - 11°C (352°C - 443°C)
352°C
410°C
Wt. of smoke (as percentage wt. of charge)
Percentage of volatile matter (D.M.F.)
0 4 8 12 16 20 24 28 32 36 40 44
1 2 3 4 5 6 7

Fig. 1. Variation of smoke with volatile matter: comparison between a series of chars and a range of bright coals

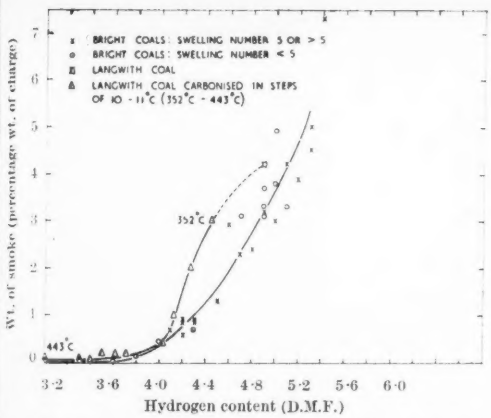

BRIGHT COALS: SWELLING NUMBER 5 OR > 5
BRIGHT COALS: SWELLING NUMBER < 5
LANGWITH COAL
LANGWITH COAL CARBONISED IN STEPS OF 10 - 11°C (352°C - 443°C)
352°C
443°C
Wt. of smoke (percentage wt. of charge)
Hydrogen content (D.M.F.)

Fig. 2. Variation of smoke with hydrogen content: comparison between a series of chars and a range of bright coals

In Fig. 2 the smoke emission from chars prepared from a low-rank coal is also plotted against their hydrogen contents and the curve obtained may be compared with that from the range of coals discussed above. At the lowest carbonizing temperatures (352–382° C.) there is a rapid rate of decrease in smoke emission. As the carbonizing temperatures increase the rate of decrease decelerates. At about 382° C. (4·2 per cent hydrogen content D.M.F.) the two curves meet. Below this point the differences between the weights of smoke emitted for corresponding hydrogen values are so small they may be ignored, and for the purpose of the subsequent discussion the lower parts of the two curves may be regarded as coincident.

From differential weight losses on Langwith coal[9] it is known that its pyrolysis begins about 335° C. and the decomposition reaches a maximum rate at about 410° C. Consequently, since chars produced at 400° C. emit inappreciable amounts of smoke (Fig. 2), it would seem that the low-boiling (low molecular weight) constituents which are first evolved are the main compounds responsible for smoke formation.

Using infra-red techniques, Brown[10] examined a weakly caking and a strongly coking coal. Between 440° and 550° C. he found that hydrogen is lost by the removal of edge groups and the evaporation of small molecules followed by the loss of aromatic hydrogen and some graphitization at higher temperatures.

The rank of coal[11] as well as the temperature of carbonization will affect the rate and type of decomposition and the structure of the residue. These factors will obviously influence the constitution of the residue and the composition and amount of smoke evolved from a particular char during combustion. The results with chars from Langwith coal may therefore be used only cautiously when coals of other rank are considered.

When volatile matter is the abscissa and smoke the ordinate (Fig. 1) then the curve for chars lies below that for coals, but when hydrogen content is the abscissa (Fig. 2) the positions of the two curves are reversed.

The explanation proposed is that although a char and a coal may yield the same volatile matter in the B.S. test, the constituents evolved may be quite different. The coals will evolve moisture and low-molecular weight hydrocarbons which react and polymerize to form "tarry bodies"[12], whereas the chars will evolve hydrogen and other gases which ignite more easily or react together and condense less readily than those from coals. Consequently, a coal with the same volatile matter as a char will produce more smoke. It is not clear why a char with a hydrogen content equivalent to that of a coal yields more smoke.

The laboratory work has suggested that either the tar yield or the hydrogen content provides a better indication of the amount of smoke emitted from coals than does the volatile matter. Other work now in progress in these laboratories should indicate how far such relations hold for domestic open fires during ignition and steady-state combustion conditions.

D. FINCH


[1] Brown, R. L., *J. Inst. Fuel.*, **29**, 218 (1956).
[2] Brit. Coal Utilisation Res. Assoc. Ann. Rep., 31 (1956).
[3] Finch, D., and Raftery, M. M. (private communication), B.C.U.R.A. Information Circular No. 189 (Dec. 1957).
[4] Piersol, R. J., Illinois State Geological Survey, Rep. No. 41 (1936).
[5] Adams, W. N., Gaines, A. F., Gregory, D. H., and Pitt, G. J., *Nature*, **183**, 33 (1959).
[6] McHugo, J. W., Shaw, W. F. B., and Whittaker, D., *J. Inst. Fuel*, **28**, 318 (1955).
[7] Spodner, C. E., *J. Inst. Fuel*, **11**, 134 (1937–38).
[8] Bradbury, D. J., and Mott, R. A., *Fuel in Science and Practice*, **20**, 100 (1941).
[9] Aldridge, A. M. (private communication).
[10] Brown, J. K., *J. Chem. Soc.*, 5563, 752 (1955).
[11] Hirsch, P. B., Conference on Science in the Use of Coal, Sheffield, A, 29 (1958).
[12] Travers, M. W., *Nature*, **183**, 1321 (1959).


# TWO ENZYMIC MECHANISMS FOR HYDROGEN TRANSPORT BY PHENOLIC ŒSTROGENS


By DR. H. G. WILLIAMS-ASHMAN, M. CASSMAN and MARGARET KLAVINS

Ben May Laboratory for Cancer Research and Department of Biochemistry, University of Chicago


ŒSTROGENIC steroids such as œstradiol-17β can mediate the enzymic transfer of hydrogen between triphosphopyridine nucleotide and diphosphopyridine nucleotide[1–3]. The same enzyme concerned with this œstrogen-dependent transhydrogenation also catalyses both the reduction of these two nucleotides by œstradiol-17β, and the oxidation of their reduced forms by œstrone. There is strong evidence[1,2] that, in the transhydrogenase reaction, the steroid transports hydrogen by the change steroid alcohol ⇄ steroid ketone.

It has been suggested that this coenzymatic function of ovarian œstrogens is related to their mode of physiological action[1,2]. However, powerful œstro-

genic activity is exhibited by many phenolic compounds devoid of secondary alcoholic groups capable of reversible oxidation to ketone functions, and which fail (*a*) to act as coenzymes for hydrogen transfer in such systems[1,3,4] and (*b*) to reduce pyridine nucleotides in the presence of the enzyme which catalyses the transhydrogenation. Examples of such substances are 17-deoxyœstradiol[5], diethylstilbœstrol and hexœstrol[6], doisynolic and allenolic acids[7], and *iso*flavones such as genistein[8]. Accordingly, it became of interest to examine model enzymic systems for the transport of hydrogen by phenolic œstrogens of this nature. Hochster and Quastel[9] observed that, in the presence of manganese dioxide as a terminal hydrogen acceptor, diethylstilbœstrol acts as a hydrogen carrier in a number of dehydrogenase systems. The quinone form of the œstrogen could be detected in the reaction mixture, and it was postulated to carry hydrogen in virtue of the reaction quinol $\rightleftarrows$ quinone. The present experiments show that both natural and synthetic phenolic œstrogens function as hydrogen carriers in two other types of enzymic reaction. The first of these is catalysed by certain phenolases and appears to involve an initial hydroxylation of the œstrogens to a corresponding *o*-diphenol; hydrogen is then transported by the change diphenol $\rightleftarrows$ quinone. The second type of reaction is catalysed by some peroxidases, accelerated by traces of manganous ions, and implicates a free-radical form of the œstrogen as a hydrogen carrier. It is well known that many simple, non-œstrogenic phenols can carry hydrogen in both enzyme systems. However, the remarkable reactivity of many phenolic œstrogens in either type of reaction emphasizes that these substances can participate directly in hydrogen transport.

Warburg[10] demonstrated that phenolases (polyphenol oxidases) are copper proteins, and that small amounts of *o*-diphenols which are oxidized by such enzymes mediate the oxidation of reduced pyridine nucleotides[10,11]. We have found that phenolases purified from white potatoes and edible mushrooms oxidize reduced di- or tri-phosphopyridine nucleotides on the addition of trace amounts of many phenolic œstrogens. The oxidations proceed to completion with the consumption of one atom of oxygen per mole of reduced pyridine nucleotide oxidized. Fig. 1 shows that with œstradiol-17β as carrier, a definite induction period occurs before the rate of oxidation of reduced diphosphopyridine nucleotide reaches a maximal value, whereas with the corresponding *o*-diphenol 1,3,5-œstratriene-3,4, 17β-triol[12], no such lag is observed. Hexœstrol and 3-hydroxyhexœstrol behave in an analogous manner. The activity of the mushroom and potato enzymes in these reactions parallels their phenolase activity (measured by the oxidation of tyrosine plus 3,4-dihydroxyphenylalanine) during fractionation procedures which result in purifications of more than fifty-fold. The œstrogen-stimulated oxidation of reduced pyridine nucleotide is unaffected by catalase, but is abolished by heating the enzymes to 70° for 10 minutes, and by the addition of 0·001 *M* sodium cyanide. The cyanide-inhibited enzyme can be reactivated by cupric ions. Œstradiol-17β did not mediate the oxidation of reduced diphosphopyridine nucleotide in the presence of hæmocyanin, of copper sulphate (0·0001 *M*) or of the soluble phenolase of spinach leaves.

A free phenolic hydroxyl group is essential for both natural and synthetic œstrogens to transport hydrogen by these phenolase-catalysed reactions.

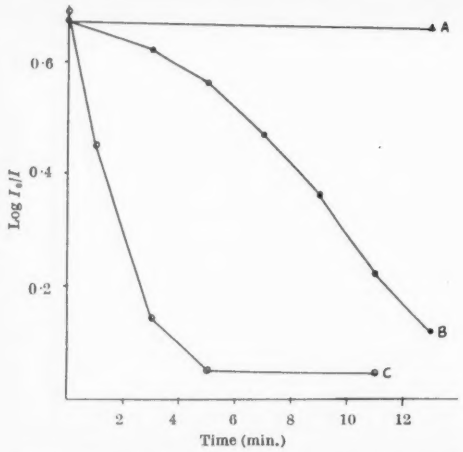



Fig. 1. Oxidation of reduced diphosphopyridine nucleotide by phenolase in the presence of potato phenolase. Sodium phosphate buffer *p*H 7·4 (0·05 *M*), 15 μgm. œstrogenic phenol in 0·01 ml. dioxane; 200 μgm. potato phenolase; 0·3 μmoles DPNH. Total vol. 3 c.c. Light path 1 cm. Wave-length 340 mμ. 25° C. In air. *A*, no œstrogen; *B*, œstradiol 17β; *C*, 1, 3, 5, œstratriene- 3, 4, 17β triol

Thus, nearly equivalent carrier activity is found with œstradiol-17α or -17β, 17-deoxyœstradiol, œstrone and œstriol, while 3-deoxyœstradiol-17α or -17β are completely inert. *bis*-Dehydrodoisynolic acid is a good hydrogen carrier, whereas the corresponding O-methyl ether is inactive. At least one phenolic hydroxyl group must be present in analogues of diethylstilbœstrol and hexœstrol[13] to exert this carrier function. The activity of natural and synthetic œstrogens is affected markedly by minor structural changes in the molecule. Thus, 1-methyl-œstradiol-17β, 2-nitro-œstrone and 4-nitro-œstrone will not act as hydrogen carriers, and are without influence upon the action of œstradiol-17β. 6β-Hydroxyœstradiol-17β (which does not mediate the oxidation of reduced diphosphopyridine nucleotide) and 7-keto-œstrone (which has approximately 10 per cent of the activity of œstradiol-17β), both at equimolar concentrations, depress the hydrogen-transporting activity of œstradiol-17β. The concentrations of œstrogens permitting 50 per cent of the maximal rate of oxidation of reduced diphosphopyridine nucleotide by potato phenolase were found to be $1 \times 10^{-6}$ *M* for 1,3,5-œstratriene-3,4, 17β-triol; $3 \times 10^{-6}$ *M* for œstradiol-17β, œstrone, 17-deoxy-œstradiol and hexœstrol, and $1 \times 10^{-5}$ *M* for genistein.

Hydrogen transport by phenolic œstrogens under these conditions can be described as follows. The induction period with monophenolic œstrogens probably reflects the time required for the phenolase to catalyse hydroxylation to *o*-diphenol derivatives. The diphenol is oxidized by the phenolase to the corresponding quinone, and the quinone is then reduced by the reduced pyridine nucleotide. Although it is not known whether the latter reaction is enzymically catalysed or not, it may be mentioned that pyridine nucleotide-menadione[14] and -quinone[15] reductase activity is readily separable from the œstrogen-mediated reactions during purification of the phenolases. In accordance with this formulation it was found that, after aerobic incubation with potato phenolase, diphosphopyridine nucleotide, ethanol and crystalline yeast alcohol dehydrogenase,

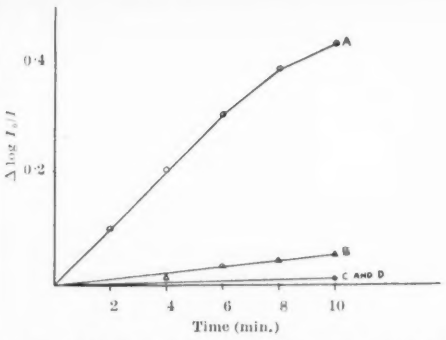



Fig. 2. Oxidation of reduced diphosphopyridine nucleotide by peroxidase in the presence of œstrogenic phenols. *Tris* (hydroxymethyl) amino methane buffer (0·03 *M*) *p*H 7·4; 0·3 μmole DPNH; 20 μgm. œstradiol-17β in 0·02 ml. ethanol; 0·03 μmole $MnCl_2$; 5 μgm. horseradish peroxidase. Total vol. 3 c.c. Light path 1 cm. 25° C. in air. Wave-length 340 mμ. *A*, complete system; *B*, omit œstradiol-17β; *C* and *D*, omit $MnCl_2$ or œstradiol-17β and $MnCl_2$

œstradiol-17β is converted to at least two other substances which migrate much more slowly than œstradiol-17β when chromatographed on paper with a heptane–methanol solvent[16].

These findings are undoubtedly related to the reported inactivation of œstrogens by plant phenolases[17]. It is possible that they have some bearing on the formation of hydroxylated derivatives of œstradiol-17β catalysed by liver microsomes in the presence of reduced triphosphopyridine nucleotide and oxygen[18], the stimulation of formate incorporation into isolated uterus by the addition of *o*-hydroxylated derivatives of œstradiol-17β *in vitro*[12], and the urinary excretion of 2-methoxy forms of œstrone[19] and œstriol[20].

An entirely different type of hydrogen transport mediated by phenolic œstrogens is catalysed by peroxidases purified from either horse radish or cow's milk. Fig. 2 shows that reduced diphosphopyridine nucleotide is oxidized upon the addition of peroxidase and low concentrations of œstradiol-17β, and that the reaction is stimulated by $10^{-5}$ *M* manganous chloride. Manometric experiments revealed that the oxidation of reduced diphosphopyridine nucleotide proceeds to completion with the consumption of one atom of oxygen per mole of reduced diphosphopyridine nucleotide oxidized. Reduced triphosphopyridine nucleotide and reduced nicotinamide mononucleotide are oxidized at the same rate as reduced diphosphopyridine nucleotide. This phenol-dependent oxidation of reduced pyridine nucleotide does not require the addition of hydrogen peroxide. Under these experimental conditions, hydrogen peroxide does not induce the oxidation of reduced diphosphopyridine nucleotide unless an appropriate phenol is present. With œstradiol-17β, or hexœstrol or diethylstilbœstrol as co-factors, the oxidation of reduced diphosphopyridine nucleotide is abolished by 0·001 *M* sodium cyanide and by catalase. 50 per cent of the maximal rate of oxidation was found with a final concentration of œstradiol-17β of $8 \times 10^{-6}$ *M*. A free phenolic hydroxyl group is required for œstrogens to exert a carrier function. In contrast to the phenolase-catalysed reactions described above, no induction period is observed when monophenolic œstrogens mediate the oxidation of reduced diphosphopyridine nucleotide in the peroxidase system. Moreover, many *o*-diphenols which act as hydrogen carriers in the phenolase-dependent reactions are incapable of transporting hydrogen in the peroxidase system and inhibit the action of œstradiol-17β therein, for example, adrenalin, noradrenalin, and 3-hydroxytyramine. The behaviour of phenolic œstrogens under these conditions is similar to that described for a number of simple phenols by Akazawa and Conn[21]. The latter authors have pointed out the similarity between such phenol-dependent reactions and the oxidation of dihydroxyfumaric acid catalysed by peroxidase[22]. They suggested that a ternary complex of peroxidase, $Mn^{++}$ and hydrogen peroxide catalyses the oxidation of the phenol (*R*OH) by oxygen to an oxidized form (*R*O·, presumably of a free-radical nature) and hydrogen peroxide. The latter substance could then, by the action of peroxidase, oxidize another molecule of the phenol to the oxidized (free-radical) form. Reduced diphosphopyridine nucleotide could further reduce the oxidized phenol. The process can be envisaged as:

$$2R\text{OH} + \text{O}_2 \rightarrow 2R\text{O}\cdot + \text{H}_2\text{O}_2$$
$$2R\text{OH} + \text{H}_2\text{O}_2 \rightarrow 2R\text{O}\cdot + 2\text{H}_2\text{O}$$
$$4R\text{O}\cdot + 2\text{DPNH} + 2\text{H}^+ \rightarrow 4R\text{OH} + 2\text{DPN}^+$$
$$2\text{DPNH} + 2\text{H}^+ + \text{O}_2 \rightarrow 2\text{DPN}^+ + 2\text{H}_2\text{O}$$

Our ability to recover œstradiol-17β unchanged from this peroxidase system is in agreement with this formulation.

These experiments suggest strongly that phenolic œstrogens can transport hydrogen in virtue of the reaction phenol ⇄ phenoxy radical. Electron spin resonance studies by Rexroad and Gordy[23] have shown that hexœstrol can be converted to a free-radical form(s) by irradiation. It is of interest that the injection of physiological doses of both natural and synthetic œstrogens into ovariectomized rats induces enormous increases in the activity of uterine peroxidase[24].

It is a pleasure to acknowledge many valuable discussions with Drs. Charles Huggins, Paul Talalay, Gerhard Closs, and the generous gifts of compounds from Drs. G. C. Mueller and E. V. Jensen. This work was supported by grants from the American Cancer Society, Inc.

[1] Talalay, P., and Williams-Ashman, H. G., *Proc. U.S. Nat. Acad. Sci.*, **44**, 15 (1958).
[2] Talalay, P., Hurlock, B., and Williams-Ashman, H. G., *Proc. U.S. Nat. Acad. Sci.*, **44**, 862 (1958).
[3] Villee, C. A., and Hagerman, D. D., *J. Biol. Chem.*, **233**, 42 (1958).
[4] Villee, C. A., *Cancer Res.*, **17**, 507 (1957).
[5] Huggins, C., and Jensen, E. V., *J. Exp. Med.*, **100**, 241 (1954).
[6] Dodds, E. C., Goldberg, L., Lawson, W., and Robinson, R., *Nature*, **141**, 247 (1938).
[7] Miescher, K., *Chem. Rev.*, **43**, 367 (1948).
[8] Bradbury, R. B., and White, R. E., *J. Chem. Soc.*, 3447 (1951).
[9] Hochster, R. M., and Quastel, J. H., *Nature*, **164**, 865 (1949).
[10] Kubowitz, F., *Biochem. Z.*, **292**, 221 (1937).
[11] Kubowitz, F., *Biochem. Z.*, **293**, 308 (1937).
[12] Mueller, G. C., *Nature*, **176**, 127 (1955).
[13] Pratt, R. J., and Jensen, E. V., *J. Amer. Chem. Soc.*, **78**, 4330 (1956).
[14] Wosilait, W. D., and Nason, A., *J. Biol. Chem.*, **206**, 255 (1954).
[15] Wosilait, W. D., and Nason, A., *J. Biol. Chem.*, **208**, 785 (1954).
[16] Dao, T. L. Y., *Endocrinology*, **61**, 242 (1957).
[17] Graubard, M., and Pincus, G., *Proc. U.S. Nat. Acad. Sci.*, **27**, 149 (1941).
[18] Mueller, G. C., and Rumney, G., *J. Amer. Chem. Soc.*, **79**, 1004 (1957).
[19] Krachy, S., and Gallagher, T. F., *J. Biol. Chem.*, **229**, 519 (1957).
[20] Fishman, J., and Gallagher, T. F., *Arch. Biochem. Biophys.*, **77**, 511 (1958).
[21] Akazawa, T., and Conn, E. E., *J. Biol. Chem.*, **232**, 403 (1958).
[22] Chance, B., *J. Biol. Chem.*, **197**, 577 (1952).
[23] Rexroad, H. N., and Gordy, W., *Proc. U.S. Nat. Acad. Sci.*, **45**, 256 (1959).
[24] Lucas, F. V., Neufeld, H. A., Utterback, J. G., Martin, A. P., and Stotz, E., *J. Biol. Chem.*, **214**, 775 (1955).

# ACTIVATION AND INHIBITION OF THE ARYLESTERASE OF HUMAN SERUM

By Prof. E. G. ERDÖS, C. R. DEBAY and M. P. WESTERMAN

Mellon Institute, Pittsburgh 13, and School of Medicine, University of Pittsburgh, Pittsburgh 13, Pa.

HUMAN blood plasma contains at least two different enzymes capable of hydrolysing phenylacetate[1,2]. One of them is a cholinesterase, the other an aromatic esterase (arylesterase)[3]. The present studies deal with arylesterase and stem from our observation that the disodium ethylenediaminetetraacetate ('Sequestrene') added to prevent coagulation in blood samples inhibited arylesterase without affecting cholinesterase activity. Since human arylesterase has been reported to be remarkably resistant to many of the usual inhibitors[2], we have supposed that further studies of the inhibition and acceleration of activity of arylesterase by selected agents would prove of interest. In particular, tests of the effects of metal ions and sequestering agents were indicated.

The activity of the enzyme was assayed with a modification of Zeller's[4] method in a Cary recording spectrophotometer at a wave-length of 2800 A. The instrument was equipped with an expanded scale (0–0·1) slide wire assembly, which greatly increased the extent of registration. The concentration of phenylacetate was $1 \times 10^{-3}$ *M*. In the early runs of the investigation, the source of enzyme was pooled, heparinized human plasma. Later, pooled, normal human serum gave similar results. The serum was diluted 1 : 2,000 v/v ; the absorption cells of the spectrophotometer contained 0·002 ml. of serum in a *tris*-hydroxymethyl aminomethane (*tris*) buffer of *p*H 7·4. At this dilution the contribution of cholinesterase to the hydrolysis of phenylacetate was found to be negligible in the sera of healthy donors. In the experiments where the effects of inhibitors or activators were tested on the cholinesterase, the source of enzyme was purified human plasma cholinesterase preparation ('Cholase', Cutter Laboratories). This preparation was void of arylesterase activity. The temperature was kept constant at 27° C. In a few control studies, the usual Warburg manometric technique or an automatic recording titrator (Titrigraph, Radiometer) gave similar results. The effect of most of the compounds on the enzyme was tested after 5-min. pre-incubation. All concentrations given in this report show the final dilution of the substance used.

It was found that the hydrolysis of phenylacetate by arylesterase increased in the presence of calcium chloride[5]. The sensitivity of the different serum samples toward calcium varied to a great extent. On the average, an 85 per cent acceleration was observed at $1 \times 10^{-4}$ *M* concentration of calcium chloride. Ethylenediaminetetraacetate also enhances the activity of arylesterase in the lower concentration ranges. This activation changes sharply to inhibition at concentrations higher than $10^{-5}$ *M*. The enzyme was totally inhibited by $2{\cdot}5 \times 10^{-5}$ *M* ethylenediaminetetraacetate. When the sodium salt of the calcium–ethylenediaminetetraacetate complex ('Sequestrene $Na_2Ca$', calcium-ethylenediaminetetraacetate) was used instead of ethylenediaminetetraacetate, no inhibition was observed. On the other hand, the magnesium – ethylenediaminetetraacetate complex inhibited similarly to ethylenediaminetetraacetate. This latter effect was probably due to the fact that calcium from the system replaced magnesium in the complex. The stability constant of ethylenediaminetetraacetate with $Ca^{2+}$ is log $K = 10{\cdot}59$ and that of $Mg^{2+}$, 8·69. Another ethylenediaminetetraacetate derivative, ethylenediamine di(*o*-hydroxyphenyl acetic acid) ('Chel *DP*'), which has little tendency to co-ordinate with calcium (log $K$ for $Ca^{2+}$ is 1·6), did not inhibit arylesterase. In some preliminary studies, swine serum arylesterase[6] was also inhibited by ethylenediaminetetraacetate.

The results obtained with calcium prompted us to investigate the effect of other cations on the enzyme. Arylesterase was inhibited by a number of rare earth, alkaline earth and metal ions. The best inhibitor was $GdCl_3$ $I_{50} = 4 \times 10^{-8}$ *M* ; the weakest, $MgCl_2$, $I_{50} = 4 \times 10^{-4}$ *M*. The inhibitory effect decreased in the following order : $GdCl_3$, $CeCl_3$, $LaCl_3$, $Y(NO_3)_3$, $SmCl_3$, $CdSO_4$, $HgCl_2$, $AgNO_3$, $PbCl_2$, $ZnSO_4$, $NiSO_4$, $CoSO_4$, $CuCl_2$, $MnSO_4$, $BaCl_2$, $SrCl_2$, $MgCl_2$.

Under the experimental conditions used, the most interesting feature of these series was the inhibition by low concentrations of the stable trivalent rare earth cations and by somewhat higher concentrations of heavy metals. Yttrium and the rare earth ions were about equally active, the $I_{50}$ values falling in the $10^{-7}$ to $10^{-8}$ *M* range. *p*-chloromercuryphenyl sulphonic acid also inhibited the enzyme ($I_{50} = 3 \times 10^{-6}$ *M*). Thus, in addition to rabbit[3], the human enzyme is also sensitive to sulphydryl agents. Sodium citrate and an amino-oxidase inhibitor, 1-phenyl-2-hydrazinopropane (*JB*516), inhibited the enzyme in a relatively high concentration. The $I_{50}$ values were $6 \times 10^{-4}$ *M* and $2 \times 10^{-4}$ *M*, respectively.

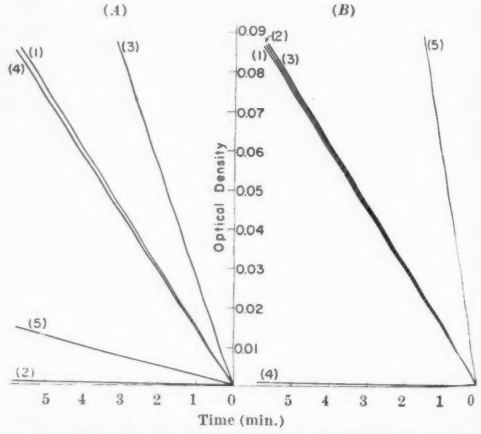



Fig. 1. Inhibition and acceleration of the hydrolysis of phenylacetate by human serum arylesterase (*A*) and by purified, concentrated human plasma cholinesterase (*B*). (1) control ; (2) ethylenediaminetetraacetate $2{\cdot}5 \times 10^{-5}$ *M* ; (3) calcium chloride, $1 \times 10^{-4}$ *M* ; (4) eserine, $2{\cdot}5 \times 10^{-6}$ *M* ; (5) *JB*516, $1 \times 10^{-3}$ *M*

As indicated above, $2 \cdot 5 \times 10^{-5}$ *M* ethylenediaminetetraacetate inhibited the enzyme completely. Presumably this inhibition is due to removing the calcium from the enzyme molecule. The acceleration by low concentrations of ethylenediaminetetraacetate may reflect a binding of an inhibitor present in the system. When a $10^{-4}$ *M* calcium chloride solution was added soon after $5 \times 10^{-5}$ *M* ethylenediaminetetraacetate to the enzyme, the inhibition gradually decreased and up to 70 per cent of the normal activity was restored. If, instead of calcium, magnesium was added, only a negligible decrease in inhibition was observed.

When a 1 : 10 v/v dilution of serum was dialysed against a *tris*-buffer at 4° C., the activity of arylesterase decreased 65 per cent within 17 hr., but it could be partially restored by adding calcium to the dialysed enzyme. If the enzyme solution contained ethylenediaminetetraacetate in the dialysis tube, 95 per cent of the activity disappeared during the dialysis and calcium did not reactivate the enzyme. This indicates that the removal of calcium from the arylesterase led to irreversible changes. When the enzyme solution contained calcium-ethylenediaminetetraacetate during the dialysis instead of ethylenediaminetetraacetate, the enzyme activity remained the same as that of the dialysed control. Finally, when the enzyme was dialysed against a *tris*-buffer which contained $10^{-4}$ *M* calcium chloride, the activity did not diminish during dialysis.

Others have shown earlier that phenylacetate is hydrolysed by both arylesterase and the cholinesterase of human serum or plasma. However, Fig. 1 summarizes some of the differences between the two enzymes. In this figure the increase in optical density is plotted against time in minutes. This increase is due to the amount of phenol liberated which in turn is a function of the enzymic activity. Part *A* shows that arylesterase was inhibited by ethylenediaminetetraacetate and *JB*516, activated by calcium, and unaffected by eserine[1–3]. Cholase (part *B*), as expected, was inhibited by eserine. The activity of cholinesterase with phenylacetate substrate was accelerated by *JB*516 and unaffected by $1 \times 10^{-4}$ *M* $Ca^{2+}$ and ethylenediaminetetraacetate.

The results summarized in this report indicate the dependence on calcium of the arylesterase as well as the sensitivity of this enzyme toward several inhibitors. A detailed account of the work with arylesterase and on the effect of *JB*516 on cholinesterase will be published elsewhere.

Ethylenediaminetetraacetate, its derivatives, and the technical data were kindly supplied by Geigy Industrial Chemicals; *JB*516 by Lakeside Laboratories.

[1] Mounter, L. A., and Whittaker, V. P., *Biochem. J.*, **54**, 551 (1953).
[2] Augustinsson, K. B., *Nature*, **181**, 1786 (1958).
[3] Aldridge, W. N., *Biochem. J.*, **53**, 110 (1953); **53**, 117 (1953); **54**, 442 (1953).
[4] Zeller, E. A., *Arch. Biochem. Biophys.*, **61**, 231 (1956).
[5] Bernsohn, J., Zeller, E. A., and Cochrane, L. S. G., *Fed. Proc.*, **13**, 183 (1954).
[6] Augustinsson, K. B., and Olsson, B., *Biochem. J.*, **71**, 477 (1959); **71**, 484 (1959).

# REDUCTION OF TOXICITY OF CATIONIC MACROMOLECULES BY COMPLEXING WITH ANIONIC DERIVATIVES OF SYNTHETIC POLYGLUCOSES

By P. T. MORA, B. G. YOUNG and M. J. SHEAR
National Institutes of Health, Bethesda 14, Maryland

ANIONIC derivatives of synthetic polyglucoses were found[1,2] to inhibit enzyme activity of cationic proteins in consequence of the formation *in vitro* of complexes through electrostatic forces. In the case of lysozyme, small amounts of salt prevented complex formation; when added after complexing, salt caused dissociation accompanied by reversal of the inhibition. On the other hand, low salt concentration did not prevent complexing with ribonuclease, hyaluronidase and some other enzymes.

The present communication reports experiments designed to ascertain whether the toxicity of cationic drugs of high molecular weight can be effectively blocked by analogous complexing with anionic polyglucose derivatives in the living animal, where complex formation can be affected by the salt and by the competing cationic macromolecules which are present. It was found that the toxicity of a number of such materials could, indeed, be reduced. Our findings extend the recent report of Higginbotham[3] that heparin, a naturally occurring anionic polysaccharide, reduced the toxicity of polymyxin-*B* in mice.

In the current experiments the compounds were employed in solution in pyrogen-free water; the total volume of fluid administered was kept between 0·1 and 0·4 ml. per animal. Ten-week old strain *C* mice of both sexes, weighing 18–20 gm., were employed in groups of ten. The mice were observed for several hours after treatment, and 24-hr. survival was tabulated.

The cationic substances administered at toxic level were polymyxin-*B*, protamine, streptomycin, and neomycin. Toxic effects were found to be reduced by subsequent administration of the sodium salts and of the free acid forms of the sulphate and carboxyl derivatives of polyglucose. Two sulphated preparations were employed, both derived from a polyglucose with a number average molecular weight of about 20,000; the number of sulphate groups per anhydro-glucose unit was 3 and 0·6, respectively. The former was preparation *H*[4] $[\eta] = 0 \cdot 04$; the latter was preparation *D*[4], $[\eta] = 0 \cdot 04$; the free acid form was obtained by treatment with a cation exchange resin. The carboxyl derivative contained 17 per cent carboxyl (preparation *e*)[5].

Polymyxin-*B* was given in a standard dose of 0·5 mgm./mouse. The mice reacted as follows: within 3 min. they were prostrated; respiratory embarrassment and severe convulsions developed in about 10 min.; they began to die in about 20 min.; those few that survived for 40 min. recovered and were still alive at 24 hr.

Table 1. Counteraction of Toxicity of Cationic Macromolecules by subsequent Administration (5 min. later) of Anionic Polyglucose Derivatives

| Toxicity from | | Counter treatment | | 24-hr. survival (10 mice per group) |
|---|---|---|---|---|
| Cationic substance | Mgm./mouse | Polyglucose derivative | Mgm./mouse | |
| Polymyxin-*B* | 0·5 | — | — | 2 |
| | | Sulphate *H* (acid form) | 1 | 10 |
| | | Sulphate *H* | 0·5 | 10 |
| | | Sulphate *H* | 1 | 9* |
| | | Sulphate *H* | 1 | 8† |
| | | Sulphate *H* (Na salt) | 1 | 10 |
| | | Carboxyl (acid) | 1 | 10 |
| | | (acid) | 0·5 | 10 |
| | | (Na salt) | 0·5 | 10 |
| Protamine | 3 | Control | — | 0 |
| | | Sulphate *H* (acid) | 3 | 10 |
| Streptomycin | 8 | Control | — | 2 |
| | | Sulphate *H* (acid) | 8 | 8 |
| Neomycin | 3 | Control | — | 4 |
| | | Sulphate *H* (acid) | 6 | 8 |
| | | | 10 | 6 |

* 10 min. interval between injections.
† 20 min. interval between injections; two mice were already dead.

When polyglucose sulphate was administered 5 min. after this dose of polymyxin-*B*, all the mice recovered rapidly and behaved normally within 30 min.; even when the counteracting dose was delayed for 10 or 20 min., at which time some of the mice were already dead, the moribund animals recovered. Similar counteraction of the toxicity of polymyxin was obtained with both the salt and the acid forms of the sulphate and carboxyl derivatives (Table 1). Analogous protection was obtained against the lethal effect of protamine. However, the toxicity of streptomycin and of neomycin was only partially reduced even when larger amounts of polyglucose sulphate were used. This is in line with the requirement for high molecular weight in the blocking[2] of enzyme activity.

In the second set of experiments (Table 2) the polyglucose derivative was injected first, subcutaneously at the nape, while the cationic substance was given intraperitoneally. In the experiments with polyglucose sulphate and polymyxin-*B*, protection against lethality and toxic manifestations was virtually complete when the interval between the injections was 2–90 min. The protective effect of the carboxyl derivative was of shorter duration. Polyglucose sulphate (1 mgm.) yielded partial protection against the lethal dose of protamine (3 mgm.).

Table 2. Protection from Lethal Toxicity of Polymyxin-*B* (0·5 mgm./mouse) by prior Injection of Anionic Polyglucose Derivatives at a Different Site

| Protective treatment | | Time between injections | 24-hr. survival (10 mice per group) |
|---|---|---|---|
| Polyglucose derivative (acid forms) | Mgm./mouse | | |
| None | — | — | 2 |
| Sulphate *H* | 1 | 1 day | 4 |
| ,, | 1 | 300 min. | 3 |
| ,, | 1 | 180 ,, | 8 |
| ,, | 1 | 90 ,, | 10 |
| ,, | 1 | 60 ,, | 10 |
| ,, | 1 | 30 ,, | 10 |
| ,, | 1 | 5 ,, | 10 |
| ,, | 1 | 2 ,, | 9 |
| ,, | 0·5 | 30 ,, | 6 |
| ,, | 0·25 | 30 ,, | 5 |
| Carboxyl | 1 | 5 ,, | 10 |
| ,, | 1 | 10 ,, | 1 |
| ,, | 2 | 10 ,, | 8 |

These experiments showed that polyglucose sulphate afforded protection promptly even when injected by a different route and at a site distant from that employed for the polymyxin. In addition to the direct action of the anionic derivative upon the cationic drug, it is possible that mobilized acid polysaccharide of tissue origin also may contribute to the blocking of the lethal effect[6].

Synthetic polyglucose derivatives can provide molecular model systems suitable for the study of macromolecular interactions and of the consequent biological changes. Polyglucoses can be prepared different in molecular weight and in degree of branching[7]. They have a highly branched, spherical structure[8], and possess numerous alcoholic hydroxyl groups suitable for graded substitution with dissociating groups. For example, polyglucose sulphates with different degrees of sulphation[4] can be used to study the effect of molecular parameters (size, charge density, etc.) on macromolecular interaction[1,2].

The effect of difference in charge density of polyglucose sulphates upon their potency in counteracting the lethal effect of polymyxin-*B* was also investigated. Preparations *H* and *D* of polyglucose sulphate sodium salt, described above, were given as in the experiments summarized in Table 1, that is, the anionic derivative was injected 5 min. after the lethal dose of polymyxin-*B* (both given intraperitoneally). Table 3 shows that the polyglucose sulphate with the higher charge density gave greater protection; for example, at the 0·1-mgm. level, preparation *H* gave complete protection while preparation *D* gave none.

Table 3. Effect of Charge Density of Polyglucose Sulphate on Counteraction of Toxicity of Polymyxin-*B* (0·5 mgm./mouse injected 5 min. earlier)

| Polyglucose sulphate sodium salt | | | 24-hr. survival (10 mice per group) |
|---|---|---|---|
| Preparation | $SO_4$/anhydroglucose unit | Mgm./mouse | |
| — | — | — | 1 |
| *H* | 3 | 0·5 | 10 |
| *D* | 0·6 | 0·5 | 9 |
| *H* | 3 | 0·25 | 10 |
| *D* | 0·6 | 0·25 | 3 |
| *H* | 3 | 0·1 | 10 |
| *D* | 0·6 | 0·1 | 1 |
| *H* | 3 | 0·05 | 4 |

Thus it has been found that such anionic derivatives of polyglucose were capable of protecting mice against a lethal dose of cationic macromolecules not only when administered first, but also by counteracting toxicity in moribund animals. Furthermore, the greater their charge density the greater was their effectiveness.

Cationic polyglucose derivatives have now been synthesized. Their interaction with naturally occurring anionic macromolecules, and the effect of such interaction on the biological properties of the latter, are projected.


[1] Mora, P. T., and Young, B. G., *Nature*, **181**, 1402 (1958).
[2] Mora, P. T., and Young, B. G., *Arch. Biochem. Biophys.* (in the press).
[3] Higginbotham, R. D., and Carter, P. B., *Antibiotics and Chemotherapy*, **7**, 527 (1957).
[4] Wood, J. W., and Mora, P. T., *J. Amer. Chem. Soc.*, **80**, 3700 (1958).
[5] Mora, P. T., Merler, E., and Maury, P., *J. Amer. Chem. Soc.* (in the press).
[6] Cf. Higginbotham, R. D., *Ann. N.Y. Acad. Sci.*, **73**, 186 (1958).
[7] Mora, P. T., Wood, J. W., Maury, P., and Young, B. G., *J. Amer. Chem. Soc.*, **80**, 693 (1958).
[8] Mora, P. T., *J. Polym. Sci.*, **23**, 345 (1957).

# QUANTITATIVE ASSAY OF COMPOUNDS IN ISOLATED, FRESH NERVE CELLS AND GLIAL CELLS FROM CONTROL AND STIMULATED ANIMALS

By Prof. HOLGER HYDÉN

Department of Histology, Faculty of Medicine, University of Gothenburg

FOR cytophysiological studies it is desirable to express results in amounts of biologically important substances per cell. The same trend seems now to exist in neurocytology for unicell analyses as is the case in electrophysiology. This article gives an account and applications of the methods used in our laboratory for the determination of substances expressed as $\mu\mu$gm. per fresh nerve cell or per volume of fresh glial cells.

Lowry[1] and his associates[2] have dissected nerve cells from thick, frozen and dried sections and have obtained excellent results. We prefer to dissect nerve cells from a cut surface through the desired locus of the fresh tissue, immersed in isotonic sucrose solution. The dissection is made free-hand under a stereomicroscope, at a magnification of 64 or 100. A stainless thread, 15 or 18$\mu$ in diameter, and sharpened to $\sim 2\mu$ (manufactured by Kanthal AB, Hallstahammar, Sweden), is used for lifting out the nerve cells into an isotonic sucrose solution. The main part of the dendrites comes with the cell (Fig. 1). Usually a very small amount of methylene blue in sucrose solution is applied to the cut surface for some seconds. The stain is taken up by the synapses, which are seen as a finely dotted border around the area occupied by the unstained nerve cell. The cell is removed before it takes up the dye, and transferred to the substrate or to the sucrose solution, where it is freed from adhering glia by gentle manipulation. Note the density of synapses on the surface of the soma and dendrites of the fresh nerve cell (Fig. 1). Nerve cells sampled in this way are used for the determination of the weight,

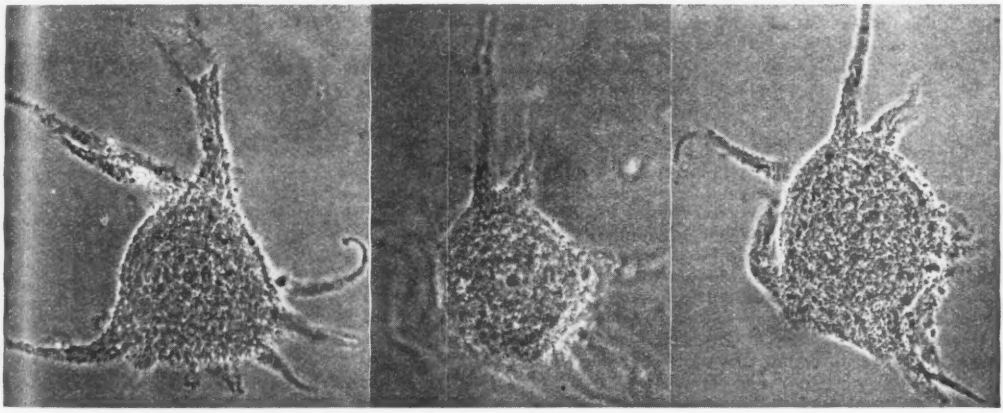


Fig. 1. Fresh nerve cells dissected out free-hand, photographed in the phase-contrast microscope. Slightly stained with methylene blue to show density of the synapses as small knobs on the surface. (× c. 200)

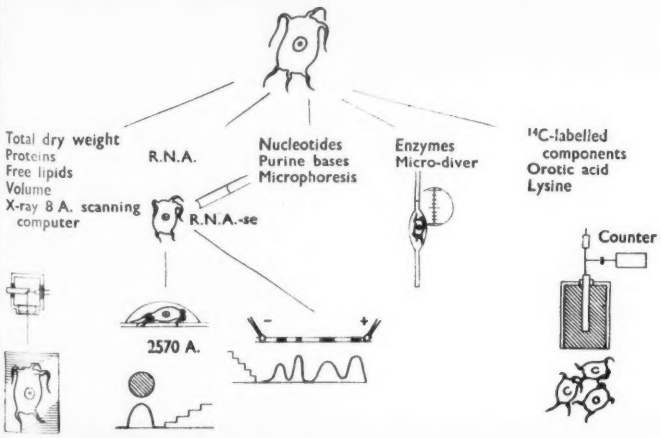



Fig. 2. Survey of the methods used on the dissected out nerve cells and their glial cells

Fig. 3. Fresh nerve cells plus the neuroglial cells originally closely surrounding each cell dissected free and trimmed to the same volume as that of their nerve cell and placed in a row below the nerve cells. The collection of glial cells slightly pressed against the glass. (Phase contrast, × *c.* 30)

a protein and a free lipid fraction[3,4], ribonucleic acid[5], nucleotides[6,7], of labelled substances such as $^{14}$C-orotic acid and $^{14}$C-lysine[8], and of enzyme activities[9] (Fig. 2). The oligodendrocytes closely surrounding the nerve cell easily come off in the substrate and adhere to each other, and the collection may assume a spherical form. It can easily be trimmed to approximately the same volume as that of the nerve cell to which they belong (Fig. 3). The collection of glial cells is freed from larger parts of axons or dendrites.

No method hitherto described is ideal for volume determination of such an irregular cell as the nerve cell; not even the interference microscope technique. The following method seems adequate. The total dry weight of a fresh nerve cell including the main part of the dendrites, precipitated with 1 *N* cold perchloric acid for 30 sec. and washed and dried, is determined by X-ray microradiography at 8–10 A. The X-radiogram is evaluated by our scanning cell analyser, which gives the weight of the cell based on up to 12,000 measurements in 4 min. The dry weight per unit volume of the nerve cell is determined on a frozen and dried section prepared in the cryostat. The volume of the fresh cell irrespective of its irregular form is the total weight divided by the mass per $\mu^3$ of the cell material. The value for the organic material in the nucleus has been found to be as high as that in the cytoplasm.

It was found that the mass per unit volume of fresh glial cells is 0·20 $\mu\mu$gm./$\mu^3$, which is the same as that of their nerve cells. Hence, it is possible to compare nerve cells and glial cells on the same volume basis.

Rabbits were subjected to mild rotation, through 120° horizontally and 30° vertically, 35 turns/min., for 25 min./day for 6 days. The results—those on the effect of stimulation on the nerve cells—will be published in detail elsewhere, in collaboration with Dr. A. Pigon.

Table 1. Data from 80 Deiter's Nerve Cells, Rabbit
Values in $\mu\mu$gm. Vol. averaged 93·200 $\mu^3$. The dry weight as $\mu\mu$gm./$\mu^3$ was 0·23

| Structure | Dry weight | Proteins | Lipids soluble in chloroform | Ribonucleic acid | Ribonucleic acid in per cent of dry proteins |
|---|---|---|---|---|---|
| Soma | 20,800 | 16,600 | 4,200 | 1,100–1,300 | 7 |
| Nucleoplasm | 800 | 650 | 200 | ~20 | |
| Nucleolus | 100 | | | | |
| Nerve cell total | 21,700 | 17,250 | 4,400 | | |

The ribonucleic acid content of the oligodendrocytes expressed per volume, equivalent to that of the nerve cell, was in 15 analyses found to be approximately 100 $\mu\mu$gm., that is, ten times less than the ribonucleic acid content of the nerve cell. This is noteworthy since the number of glial cells surpasses that of the neurons by a factor of probably more than ten. Thus, it is not possible to state that ribonucleic acid found in bulk analyses of the central nervous system is that of the nerve cells, as is stated in several papers.

Table 2. Effect of Vestibular Stimulation on the Dry Weight of Deiter's Cells, Rabbit
Values in $\mu\mu$gm.

| Nerve cells | No. | Weight | *V*† (per cent) | *P* |
|---|---|---|---|---|
| Controls | 50 | | 19 | 0·01** |
| Stimulated 25 min./day for 6 days | 47 | 24,300 | 31 | |

† *V*, variation coefficient.

The effect of the vestibular stimulation on these cells, second in the neuronal chain, is thus a significant increase of the dry weight of organic material. The conclusion is that the increased functional demands cause a production of intraneuronal organic material, the proteins included. This fits with earlier findings using another type of technique[10].

With measurements also on nerve cell plus same volume of glial cells altogether 500 cells have been measured.

In the controls, the respiratory enzyme activity is twice as high in the glial cells as in the nerve cell they belong to, measured on the same volume. This fits well with the results of the same enzyme activity in spinal ganglion cells and in their satellite cells[9],

Table 3. Effect of Vestibular Stimulation on the Respiratory Enzyme Activity in Deiter's Cells and in the same volume of their Glial Cells
Enzyme activity as 10$^{-4}$ $\mu$l. $O_2$/hr./cell at 37°. Cells measured one, two or three at a time in one micro-diver

| | Cytochrome oxidase | | | | Succinoxidase | | | |
|---|---|---|---|---|---|---|---|---|
| | No. of cells | Mean | *P* | Ratio | No. of cells | Mean | *P* | Ratio |
| Nerve cells, stimulated | 25 | 7·6 | 0·01** | 1·8 | 57 | 6·0 | 0·001*** | 2·8 |
| Nerve cells, controls | 34 | 4·2 | | | 35 | 2·1 | | |
| Glial cells, stimulated | 36 | 3·0 | 0·001*** | 0·3 | 41 | 4·0 | 0·6 | 0·9 |
| Glial cells, controls | 34 | 11·5 | | | 33 | 4·5 | | |

and also with respect to other enzyme activities in this type of nerve cells[11].

The vestibular stimulation for 25 min./day for 6 days increased the respiratory enzyme activity of the nerve cells, belonging to the second neuron of this pathway, to a higher level. Considering that the organic mass per nerve cell also increased, it may be assumed that the stimulation caused an increase in the enzyme concentration as well as activity. But, at the same time, the enzyme activity in the glial cell decreased, and significantly so in the case of cytochrome oxidase. It is impossible at present to interpret these results. The following tentative working hypothesis may be presented. Several observations support the view that the glial cells, especially the oligodendrocytes, are donors of energy or of food to the nerve cell they serve. It is also known that there occurs a shift in the substrate for the metabolism of the stimulated central nervous system compared with the non-stimulated. The central nervous system can furthermore burn substances other than glucose[12].

Therefore, when the nerve cells demand more energy at the increased activity, they are presumably given priority to the necessary substances by the neuroglia. These cells may cut down their metabolism, which is reflected in the decreased cytochrome oxidase activity. There may be an inhibiting mechanism at that terminal step, at increased neuronal activity, and the glial cells may be the cellular parts of the central nervous system which have to resort to substances other than glucose.

These studies have been supported by the Rockefeller Foundation and by the Swedish Medical Research Council.

[1] Lowry, O. H., *J. Histochem. Cytochem.*, **1**, 420 (1953).
[2] Robins, E., *Exp. Cell. Res.*, Supp. 4, 241 (1957).
[3] Brattgård, S. O., and Hydén, H., *Int. Rev. Cytol.*, **3**, 455 (1954).
[4] Hydén, H., Proc. IV Int. Cong. of Biochemistry, Symp. III, 1 (1958).
[5] Edström, J.-E., *J. Neurochem.*, **3**, 100 (1958).
[6] Edström, J.-E., and Hydén, H., *Nature*, **174**, 128 (1954).
[7] Edström, J.-E., *Microchem. J.*, **2**, 71 (1958).
[8] Brattgård, S. O., Hydén, H., and Sjöstrand, J., *Nature*, **182**, 801 (1958).
[9] Hydén, H., Lavtrup, S., and Pigon, A., *J. Neurochem.*, **2**, 304 (1958).
[10] Hamberger, C.-A., and Hydén, H., *Acta Oto-Laryngol.*, Supp. 75, 53, 82 (1949).
[11] Lowry, O. H., in "Progress in Neurobiology II: Ultrastructure and Cellular Chemistry of Neural Tissue", ed. H. Waelsch, 69 (Hoeber-Harper, New York, 1957).
[12] Geiger, A., "Metabolism of the Nervous System", ed. D. Richter, 245 (Pergamon Press, London, 1957).

# VIRAL MULTIPLICATION AND CELLULAR HYPERPLASIA

By Prof. COUNCILMAN MORGAN
Department of Microbiology, College of Physicians and Surgeons, Columbia University, New York, 32

In assessing the mechanisms whereby certain viruses cause either cellular proliferation or neoplasia, it is obviously important to determine whether replication of the virus augments or inhibits mitosis of the host cell. Data bearing directly on this problem are scant. Dawson[1], studying the histology of focal lesions in chicken chorioallantoic membranes infected with herpes simplex virus, reported necrosis and hyperplasia of the ectodermal cells but found that only "some of the cells show the characteristic intranuclear changes of infection". Burnet *et al.*[2] confirmed these observations and noted that the proliferation followed initial necrosis of the ectoderm. In addition, these authors described hyperplasia of the entodermal cells without the nuclear swelling, margination of chromatin and formation of inclusion bodies which were believed to accompany viral infection. Later, Beveridge and Burnet[3] found inclusion bodies "only with great difficulty" in some chorioallantoic lesions induced by herpes simplex virus. The foregoing observations, together with the fact that sterile broth, saline and emulsions or filtrates prepared from animal tissue caused proliferation of the ectoderm at the site of inoculation, led the authors to suggest that in the case of viral infection "the cell is damaged, and in response to something diffusing from the damaged cell—either virus particles or, more probably, growth stimulating substances resulting from primary damage—neighbouring ectodermal cells proliferate".

Regarding neoplasms, it has been recognized that in the human skin some verruca caused by viruses[4] were characterized by the presence of intranuclear inclusion bodies[4,5] and by the appearance in the electron microscope of intranuclear crystalline arrays of particles presumed to represent virus[6]. Bunting *et al.*[7] and Blank[8], however, noted that the cells which contained the inclusion bodies were not seen in process of division. More recently, Bloch and Godman[9] reported that the bulk of these tumours was composed of "normal appearing cells" and suggested that "epidermal cells with morphological stigmata of infection, that is inclusion bodies, have lost their capacity for mitotic proliferation". These authors proposed either that the explanation by Beveridge and Burnet regarding growth-promoting substances was correct or that the viral infection was present but morphologically and cytochemically inapparent in the light microscope.

The preceding studies by light microscopy were largely dependent upon the recognition of inclusion bodies as presumptive evidence for the presence of virus. It has not been definitely established, however, what relationship the inclusion bodies bear to the viral particles themselves, nor is it known whether viral development is invariably accompanied or followed by the appearance of an inclusion body in the host cell. Another approach to the problem of identifying virus in specific cells was provided by the fluorescent antibody technique. Noyes and Mellors[10] examined sections of rabbit papillomas stained with fluorescent antibody to the antigen of the Shope papilloma virus. The antigen was found to be intranuclear and generally confined to cells of the superficial keratohyaline or keratinized layers. "It was surprising," the authors commented, "that practically all the cells in the proliferating layers of the papilloma contained no antigen, for the cells in these layers make the major contribution to the growth of the papilloma." They added that "there was failure to demonstrate any viral antigen in the mitotic figures of the proliferating layers". From these results the

authors advanced the postulate "that the virus is present in the germinal and the proliferating cells but exists there in an early stage of development, consisting mainly of nucleic acid and deficient in protein, and therefore non-antigenic and not demonstrable by fluorescent antibody". The possibility that virus was present but in insufficient amounts to produce fluorescence could not be excluded.

With the advent of techniques suited to the preparation of thin sections for electron microscopic examination, a method was at hand for visualizing viruses in host cells at sufficient resolution to detect stages in the differentiation of structural components. Of the viruses mentioned above, herpes simplex virus lends itself most readily to study in the electron microscope by virtue of the fact that it grows well both in tissue cultures and in the chorioallantoic membrane. Employing the latter cell system, it had been determined previously[11] that the virus develops in the nucleus where it differentiates within characteristic aggregates of granules and appears initially as a central body enclosed by a single membrane. Afterwards, a second membrane is formed. Particles with a central body and two peripheral membranes appear to be the completed, infectious unit. Once the stages in morphological development were recognized it became possible to identify an infected cell with considerable certainty simply by the presence of viral components, even though few in number and not fully assembled into complete virus, and by the accompanying alterations in fine structure. It was decided, therefore, to re-examine the relationship of herpes simplex virus to cellular hyperplasia. Accordingly, a recently isolated strain (*J.M.*)[12] of herpes simplex virus was transferred from tissue cultures directly to chorioallantoic membranes. Three and five days after inoculation the resulting focal lesions were fixed in osmium tetroxide, dehydrated in ethyl alcohol, embedded in methacrylate and cross-sectioned at multiple levels for examination in the electron microscope. Thick sections were also cut and stained for orientation by light microscopy. At three days there was marked hyperplasia of the ectoderm with necrosis of the superficial cells toward the centre of the lesion. The mesoderm showed œdema and infiltration by inflammatory cells and there was moderate, but definite, hyperplasia of the underlying entoderm. The most striking feature revealed by the electron microscope was that only a small proportion of the ectodermal, epithelial cells contained viral components or, indeed, showed any of the characteristic changes in nuclear fine structure associated with viral multiplication. At five days more cells of the ectoderm were found to be infected and necrosis had extended. Contrary to expectation, however, many of the cells undergoing necrosis were devoid of virus. In no instance was an infected cell encountered within the hyperplastic entoderm. The foregoing suggests that under the conditions of these experiments *hyperplasia is not dependent upon the intracellular* presence of recognizable virus or viral components.

Recently, Stoker[13], studying single HeLa cells in tissue cultures infected with herpes simplex virus, found that "none of the cells which yielded virus divided" and, further, that using tissue cultures in synchronous division it was possible to inhibit mitosis by the addition of virus even 1 hr. before division was to occur. If it could be shown that the mode of viral development were similar both in tissue culture and in the chorioallantoic membrane, it would not be unreasonable to assume that Stoker's observations regarding the inability of infected cells to divide would apply to the latter cell system as well. Consequently, detailed comparison was made by electron microscopy between the development of the *J.M.* strain in chorioallantoic membranes and its development in HeLa and human amnion cells grown in tissue culture. No basic differences either in the manner of viral evolution or in the morphological response of the host cell were encountered. Presumably, then, in the chorioallantoic membrane, as in tissue culture, herpes simplex virus prevents mitosis of the cells in which it differentiates. It would appear that some factor provided by the intact host is necessary for the hyperplasia to occur, for, as Stoker[13] has pointed out, there is little evidence to suggest that this phenomenon is associated with the infection of tissue cultures by herpes simplex virus. There are insufficient data to determine whether virus also interferes with mitosis of host cells in the case of tumours, but the observations cited above regarding verrucæ and the Shope papilloma are certainly consistent with such a concept. In this connexion, it is of interest that the virus encountered in the Lucké carcinoma of frogs[14] closely resembles herpes simplex virus in morphology and intranuclear site of multiplication.

The purpose of this communication has been to indicate that the hypothesis advanced by Beveridge and Burnet regarding the hyperplasia associated with infections by herpes simplex virus is supported by electron microscopic examination and warrants further study with the techniques currently available. Although none of the observations herein reported exclude the possibility that a masked form of the virus is operative in neoplasia, it is equally possible that in certain neoplasms a cellular product other than the virus itself stimulates mitosis. The role, then, of the initiating viral infection would be to generate or liberate this product. If such were the case, it would explain the apparent contradiction presented by the suggestion that a virus may inhibit mitosis, on one hand, and stimulate cellular division, on the other, for the virus could multiply at a site removed from the region of cellular proliferation. Moreover, the wide variation encountered in viral assays of tumours[15] could be accounted for if the stimulus for mitosis were mediated not by the virus *per se*, but by a cellular product resulting from viral infection. In these circumstances the titre of virus within the tumour would not necessarily bear a consistent relationship to the extent of mitotic activity.

This study was aided by a grant from the National Foundation.


[1] Dawson, J. R., *Amer. J. Path.*, **9**, 1 (1933).

[2] Burnet, F. M., Lush, D., and Jackson, A. V., *Aust. J. Exp. Biol. Med. Sci.*, **17**, 35 (1939).

[3] Beveridge, W. I., and Burnet, F. M., Med. Res. Council (Great Britain), Special Rep. Ser. No. 256 (1946).

[4] Lyell, A., and Miles, J. A. R., *Brit. Med. J.*, **1**, 912 (1951).

[5] Strauss, M., Bunting, H., and Melnick, J., *J. Inf. Dermat.*, 15, 433 (1950).

[6] Bunting, H., *Proc. Soc. Exp. Biol. Med.*, **84**, 327 (1953).

[7] Bunting, H., Strauss, M., and Banfield, W., *Amer. J. Path.*, 28, 985 (1952).

[8] Blank, H., *Ann. New York Acad. Sci.*, **54**, 1226 (1952).

[9] Bloch, D. P., and Godman, G. C., *J. Exp. Med.*, **105**, 161 (1957).

[10] Noyes, W. F., and Mellors, R. C., *J. Exp. Med.*, **106**, 555 (1957).

[11] Morgan, C., Ellison, S. A., Rose, H. M., and Moore, D. H., *J. Exp. Med.*, **100**, 195 (1954).

[12] Morgan, C., Jones, E. P., Holden, M., and Rose, H. M., *Virology*, 5, 568 (1958).

[13] Stoker, M. G. P., Ninth Symp. Soc. Gen. Microbiol., 142 (Cambridge Univ. Press, London, 1959).

[14] Fawcett, D. W., *J. Biophys. Biochem. Cytol.*, **2**, 725 (1956).

[15] Beard, J. W., *Cancer Res.*, **16**, 279 (1956).

# FORTHCOMING EVENTS

### Saturday, October 3

ASSOCIATION OF CLINICAL BIOCHEMISTS (joint meeting with the Association of Clinical Pathologists, at the Royal College of Surgeons, Lincoln's Inn Fields, London, W.C.2), at 9.30 a.m.—Scientific Papers. 5 p.m.—Annual General Meeting.

### Monday, October 5

SOCIETY OF CHEMICAL INDUSTRY, LONDON SECTION (joint meeting with the Plastics and Polymer Group, at the Royal Institution, Albemarle Street, London, W.1), at 6.30 p.m.—Sir Robert Robinson, O.M., F.R.S.: "The Polymerisation of Olefines Using Organo-metallic Catalysts".

### Wednesday, October 7

SOCIETY FOR ANALYTICAL CHEMISTRY (at the Chemical Society, Burlington House, Piccadilly, London, W.1), at 7 p.m.—Meeting on "Atmospheric Pollution Analysis".

### Friday, October 9

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, W.C.2), at 5.30 p.m.—Sir Willis Jackson, F.R.S.—Presidential Address.

SOCIETY FOR ANALYTICAL CHEMISTRY, BIOLOGICAL METHODS GROUP (at "The Feathers", Tudor Street, London, E.C.4), at 6.30 p.m. —Discussion Meeting on "Routine Toxicity Tests in the Control of Pharmaceuticals" opened by Mr. P. Andrews.

### Saturday, October 10

NUTRITION SOCIETY (at Guy's Hospital Medical School, St. Thomas's Street, London, S.E.1), at 10.30 a.m.—Symposium on "Nutrition and the Eye".

# APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

ASSISTANT LECTURER IN AGRICULTURE—The Principal, Yorkshire (W.R.) Institute of Agriculture, Askham Bryan, near York (October 8).

HEAD OF THE DEPARTMENT OF PURE AND APPLIED PHYSICS—The Registrar, Royal Technical College, Salford 5, Lancs (October 10).

SENIOR LECTURER IN MATHEMATICS, a LECTURER IN MATHEMATICS, and an ASSISTANT, Grade "B", IN MATHEMATICS—The Registrar, Royal Technical College, Salford 5 (October 10).

SENIOR LECTURER (with particular interest in proteins or nucleic acids) IN THE DEPARTMENT OF MICROBIOLOGY—The Deputy Registrar, The University, Edgbaston, Birmingham 15 (October 14).

READER OR SENIOR FELLOW IN BIOGEOGRAPHY IN THE DEPARTMENT OF GEOGRAPHY, Research School of Pacific Studies, Australian National University, Canberra—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia, October 15).

LECTURER IN PSYCHOLOGICAL MEDICINE—The Secretary of University Court, The University, Glasgow (October 19).

CHAIR OF OPHTHALMOLOGY AT THE INSTITUTE OF OPHTHALMOLOGY —The Academic Registrar, University of London, Senate House, London, W.C.1 (October 21).

VANDERVELL CHAIR OF PHARMACOLOGY AT THE INSTITUTE OF BASIC MEDICAL SCIENCES—The Academic Registrar, University of London, Senate House, London, W.C.1 (October 22).

LECTURER IN CIVIL ENGINEERING at the University of Western Australia—*The Secretary, Association of Universities of the British* Commonwealth, 36 Gordon Square, London, W.C.1 (Australia, October 30).

CHAIR OF INORGANIC and PHYSICAL CHEMISTRY in the University of Tasmania—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia, October 31).

LECTURERS (2) IN GEOGRAPHY, and a LECTURER IN GEOLOGY at the University of Hong Kong—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Hong Kong, October 31).

TECHNICIAN in the Department of Physiological Chemistry to cut sections and apply standard histochemical tests in relation to a programme of research which is just being started—The Assistant Bursar, The University, Reading (October 31).

*FELLOW OR SENIOR FELLOW IN PHILOSOPHY within the Department* of Social Philosophy, Australian National University, Canberra—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia, November 10).

CHAIR OF ANATOMY *tenable in the Newcastle Division of the University*—The Registrar, University Office, 46 North Bailey, Durham (November 21).

CHAIR OF GEOGRAPHY in the University of Otago, Dunedin, New Zealand—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (New Zealand, December 15).

HEAD OF THE DEPARTMENT OF MATHEMATICS—The Registrar, The College of Technology, Ashley Down, Bristol 7.

# REPORTS and other PUBLICATIONS

*(not included in the monthly Books Supplement)*

## Great Britain and Ireland

Philosophical Transactions of the Royal Society of London. Series A: Mathematical and Physical Sciences. No. 997, Vol. 251 (11 June 1959): The Propagation of Plane Irrotational Waves Through an Elastoplastic Medium. By L. W. Morland. Pp. 341–383. 13*s*. 6*d*. No. 998, Vol. 251 (11 June 1959): The Rational Characterization of Certain Sets of Relatively Abelian Extensions. By A. Fröhlich. Pp. 385–425. 13*s*. 6*d*. (London: Royal Society, 1959.) [186

Seventh Special Report from the Select Committee on Estimates. Session 1958–59. Treasury Control of Expenditure—(Observations of the Treasury). Pp. 16. (London: H.M. Stationery Office, 1959.) 1*s*. net. [296

Education in 1958: Being the Report of the Ministry of Education and Statistics for England and Wales. Pp. v+261. (Cmnd. 777.) (London: H.M. Stationery Office 1959.) 12*s*. net. [296

Government of Northern Ireland: Ministry of Agriculture. Leaflet No. 132: Farm Planning. Pp. 16. (Belfast: Ministry of Agriculture, 1959.) [77

Hopkin and Williams, Ltd. Organic Chemical Reagents—Monograph No. 37: Zincon: Reagent for Zinc and Mercury Metal Indicator. By Eileen A. Johnson and W. Jablonski. Pp. 6. (Chadwell Heath: Hopkin and Williams, Ltd., 1959.) [77

Royal Institute of Chemistry. Lectures, Monographs and Reports. 1959. No. 1: Recent Developments in Inorganic and Organic Analytical Chemistry. By Dr. T. S. West. Pp. ii+41. 5*s*. 6*d*. net. No. 2: Structural Analysis of Polysaccharides. By Dr. D. J. Manners. Pp. ii+39. 5*s*. net. (London: Royal Institute of Chemistry, 1959.) [77

Government of Northern Ireland: Ministry of Agriculture. Supplement to Leaflet No. 108: The Buckfast Abbey Strain of Honey Bee. Pp. 4. (Belfast: Ministry of Agriculture, 1959.) [77

General Register Office. The Registrar General's quarterly return for England and Wales—Births, Deaths and Marriages; Infectious Diseases; Weather; Population Estimates, quarter ended 31st March 1959. (No. 441, 1st Quarter 1959.) Pp. 28. (London: H.M. Stationery Office, 1959.) 2*s*. net. [77

Ministry of Labour and National Service. Industrial Health: a Survey of the Pottery Industry in Stoke-on-Trent—a Report by H.M. Factory Inspectorate. Pp. v+78. (London: H.M. Stationery Office, 1959. Published for the Ministry of Labour and National Service.) 5*s*. net. [77

Fire Protection Association. Booklet No. 24: Industrial Solvents and Flammable Liquids. Revised, new edition. Pp. 24. (London: Fire Protection Association, 1959.) [77

Commonwealth Education: The United Kingdom Contribution. Prepared for the Commonwealth Relations Office and the Colonial Office by the Central Office of Information. Text by Duncan Crow. Pp. 68+4 plates. (London: H.M. Stationery Office, 1959.) 2*s*. 6*d*. [77

Lawes Agricultural Trust. Rothamsted Experimental Station Report for 1958. Pp. 283. (Harpenden: Rothamsted Experimental Station, 1959.) 10*s*. [77

The Coal Tar Research Association. Review of Coal Tar Technology, Vol. 10, Part 2 (July–December, 1958.) Pp. ii+87–166. (Gomersal, Leeds: Coal Tar Research Association, 1959.) 10*s*. [77

Department of Scientific and Industrial Research. Torry Research 1958 on the Handling and Preservation of Fish and Fish Products: Report of the Director of the Torry Research Station. Pp. iv+35. (Edinburgh: H.M. Stationery Office, 1959.) 2*s*. 6*d*. net. [77

## Other Countries

Laboratoire d'Astronomie de Lille. Astronomical News Letter, No. 94. Pp. i+56. Astronomical Newsletter, No. 95. Pp. i+56. (Lille: Laboratoire d'Astronomie, 1959.) [186

Smithsonian Contributions to Astrophysics. Vol. 3, No. 4: Some Sunspot and Flare Statistics. By Barbara Bell and Harold Glazer. Pp. ii+25–38. (Washington, D.C.: Government Printing Office, 1959.) 20 cents. [186

Ontario Research Foundation. Annual Report 1958. Pp. 36. (Toronto: Ontario Research Foundation, 1959.) [186

Official Records of the World Health Organization, No. 94: First Report on the World Health Situation, 1954–1956. Pp. viii+392. (Geneva: World Health Organization; London: H.M. Stationery Office, 1959.) 10 Swiss francs; 17*s*. 6*d*.; 3.25 dollars. [186

United States Department of Agriculture: Agricultural Research Service. Technical Bulletin No. 1199: Soil Fertility Studies in Lysimeters Containing Lakeland Sand. Pp. 62. (Washington, D.C.: Government Printing Office, 1959.) 25 cents. [186

Annals of the New York Academy of Sciences. Vol. 77, Article 2: Axenic Culture of Invertebrate Metazoa: a Goal. By Ellsworth C. Dougherty and 35 other authors. Pp. 25–406. (New York: New York Academy of Sciences, 1959.) 4.50 dollars. [186

Peabody Museum of Natural History, Yale University. Bulletin 13: Notes on a Collection of Birds from Mindoro Island, Philippines. By S. Dillon Ripley and D. S. Rabor. Pp. iv+82. Postilla No. 38 (April 20, 1959): Comments on Birds from the Western Papuan Islands. By S. Dillon Ripley. Pp. 17. (New Haven, Conn.: Peabody Museum of Natural History, Yale University, 1959.) [186

Commonwealth Scientific and Industrial Research Organization, Australia. Division of Fisheries and Oceanography. Technical Paper No. 5: The Barramundi *Lates calcarifer* (Bloch) in Queensland Waters. By D. J. Dunstan. Pp. 22. Technical Paper No. 6: Measurements of Light Penetration in the Tasman Sea, 1955–57. By H. R. Jitts. Pp. 26. (Melbourne: Commonwealth Scientific and Industrial Research Organization, 1959.) [186

United States Department of the Interior: Fish and Wildlife Service. Fishery Bulletin 150: Study of Age Determination by Hard Parts of Albacore from Central North Pacific and Hawaiian Waters. By Tamio Otsu and Richard N. Uchida. Pp. iv+353–363. (Washington, D.C.: Government Printing Office, 1959.) 15 cents. [186

Chicago Natural History Museum. Fieldiana: Geology. Vol. 12, No. 5: Pennsylvanian Invertebrates of the Mazon Creek Area, Illinois. By Eugene S. Richardson, Jr. Pp. i+79–82. 30 cents. Fieldiana: Zoology. Vol. 33, No. 6: Philippine Zoological Expedition, 1946–1947. Tabanidae (Diptera). By Cornelius B. Philip. Pp. i+543–625. 2 dollars. Vol. 39, No. 28: Ticks (Ixodoidea) of Arabia, with special reference to the Yemen. By Harry Hoogstraal and Makram N. Kaiser. Pp. 297–322. 65 cents. Vol. 39, No. 29: Deep Sea Fishes from the Gulf of Mexico, *Squalogadus intermedius* (Macrouroididae). By Marion Grey. Pp. 323–346. 50 cents. (Chicago: Chicago Natural History Museum, 1959.) [186

Annals of the New York Academy of Sciences. Vol. 78, Article 1: Germfree Vertebrates: Present Status. By James A. Reyniers and 30 other authors. Pp. 1–400. (New York: New York Academy of Sciences, 1959.) 5 dollars. [186

California Department of Fish and Game. Forty-fifth Biennial Report, July 1, 1956 through June 30, 1958. Pp. 95. (Sacramento: State of California, Department of Fish and Game, 1959.) [186

*European Organization for Nuclear Research. Annual Report 1958.* Pp. 87 (24 plates). (Geneva: CERN, 1959.) [186

United States Department of the Interior: Geological Survey. Bulletin 1042–S: Geology and Fluorspar Deposits, Big Four Fault System, Crittenden County, Kentucky. By George C. Hardin, Jr., and Robert D. Trace. Pp. iii+699–724+plates 61 and 62. Bulletin 1045–D: Core Logs from Bristol, Cadiz, and Danby Dry Lakes, San Bernardino County, California. By A. M. Bassett, D. B. Kupfer and F. C. Barstow. Pp. v+415–486+plates 45–48. Bulletin 1060–C: Geology and Construction-Material Resources of Pottawatomie County, Kansas. By Glenn R. Scott, Frank W. Foster and Carl F. Crumpton. Pp. iv+97–178+plate 5. Bulletin 1072–D: Quartz Crystal Deposits of Southwestern Virginia and Western North Carolina. By John B. Mertie, Jr. Pp. iv+233–298. 30 cents. Bulletin 1082–B: Radioactive Rare-Earth Deposit at Scrub Oak Mine, Morris County, New Jersey. By Harry Klemic, A. V. Heyl, Jr., A. R. Taylor and Jerome Stone. Pp. iv+29–59+plate 1. 50 cents. Bulletin 1084–B: Rapid Analysis of Chromite and Chrome Ore. By Joseph I. Dinnin. Pp. iv+31–68. 20 cents. (Washington, D.C.: Government Printing Office, 1959.) [186

United States Department of the Interior: Geological Survey. Water-Supply Paper 1353: Quality of Surface Waters of the United States 1954. Parts 9–14: Colorado River Basin to Pacific Slope Basins in Oregon and Lower Columbia River Basin. Prepared under the direction of S. K. Love. Pp. xiii+426. Water-Supply Paper 1475–F: Hydrologic Data, Wind River and Fifteen Mile Creek Basins, Wyoming, 1947–54. By Norman J. King. Pp. iv+44+6 plates. (Washington, D.C.: Government Printing Office, 1959.) [186

Bulletin of the Florida State Museum, Biological Sciences. Vol. 4, No. 4: Variation in Lizards of the *Leiocephalus cubensis* Complex in Cuba and the Osla de Pinos. By Albert Schwartz. Pp. 97–143. 55 cents. Vol. 4, No. 5: The Freshwater Decapod Crustaceans of the Apalachicola Drainage System in Florida, Southern Alabama, and Georgia. By Horton H. Hobbs, Jr., and C. W. Hart, Jr. Pp. 145–191. 60 cents. Vol. 4, No. 6: The Ostracods of the Genus *Entocythere* from the Lower Chattahoochee-Flint Basin: With a Review of the Occurrence of the Genus in Florida, and Descriptions of Two New Species. By C. W. Hart, Jr. Vol. 4, No. 7: Boarfishes of the Genus *Antigonia* of the Western Atlantic. By Frederick H. Berry. Pp. 205–250. 65 cents. (Gainesville, Fla.: Florida State Museum, 1959.) [186

Western Australia. Geological Survey Bulletin 105, Part 2: Collie Mineral Field. By G. H. Low. Pp. 135+5 plates and 13 maps. (Perth: Government Printer, 1959.) [186

United States Department of the Interior: Geological Survey. Professional Paper 305–G: Test Wells, Titaluk and Knifeblade Areas, Alaska. By Florence M. Robinson. With Micropaleontologic Study of Test Wells in the Titaluk and Knifeblade Areas, Northern Alaska, by Harlan R. Bergquist. Exploration of Naval Petroleum Reserve No. 4 and Adjacent Areas, North Alaska, 1944–53. Part 5: Subsurface Geology and Engineering Data. Pp. iii+377–422+plates 25–28. 1 dollar. Professional Paper 305–H: Test Wells, Square Lake and Wolf Creek Areas, Alaska. By Florence Rucker Collins. With Micropaleontology of Square Lake Test Well 1 and Wolf Creek Wells, Northern Alaska, by Harlan R. Bergquist. Exploration of Naval Petroleum Reserve No. 4 and Adjacent Areas, Northern Alaska, 1944–53. Part 5: Subsurface Geology and Engineering Data. Pp. iii+423–484+plates 29 and 30. 1 dollar. Professional Paper 305–J: Test Wells, Simpson Area, Alaska. By Florence M. Robinson. With a section on Core Analyses by S. T. Yuster. Exploration of Naval Petroleum Reserve No. 4 and Adjacent Areas, North Alaska, 1944–53. Part 5: Subsurface Geology and Engineering Data. Pp. iii+523–568+plates 35–38. 1.25 dollars. Professional Paper 314–G: Succession and Speciation of the Pelecypos *Astarte*. By Ralph W. Imlay. Pp. iii+155–169+plates 16–19. (Washington, D.C.: Government Printing Office, 1959.) [186

Det Kongelige Department for Industri og Håndverk. Norsk Polarinstitutt. Skrifter Nr. 116: The Algal Vegetation of Spitsbergen: a Survey of the Marine Algal Flora of the Outer Part of Isfjorden. By P. Svendsen. Pp. 49+2 plates. (Oslo: Norsk Polarinstitutt, 1959.) 7 kr. [77

U.S. Department of Commerce: National Bureau of Standards. Handbook 69: Maximum Permissible Body Burdens and Maximum Permissible Concentrations of Radionuclides in Air and in Water for Occupational Exposure—Recommendations of the National Committee on Radiation Protection. Pp. viii+95. (Washington, D.C.: Government Printing Office, 1959.) 35 cents. [77

Illinois Natural History Survey. Bulletin, Vol. 27, Article 3: Lead Poisoning as a Mortality Factor in Waterfowl Populations. By Frank C. Bellrose. Pp. ix+235–288. (Urbana, Ill.: State Natural History Survey Division, 1959.) 50 cents. [77

Bulletin of the American Museum of Natural History. Vol. 117, Article 5: A Taxonomic Revision of the Spotted Skunks (Genus *Spilogale*). By Richard G. van Gelder. Pp. 229–392. (New York: American Museum of Natural History, 1959.) 2 dollars. [77

Koninklijk Nederlands Meteorologisch Institut. Mededelingen en Verhandelingen. No. 67: Klimatologische Gegevens van de Nederlandse Lichtschepen over de Periode 1910–1940. By G. Verploegh. Part 2: Luchtdruck en Wind; Zeegand. Pp. 91. 7.50 florins. No. 73: A Theory and Method for Calculation of Wind Effects on Sea Levels in a Partly Enclosed Sea, with special application to the Southern Coast of the North Sea. By Dr. M. P. H. Weenink. Pp. viii+111. 8 florins. (*S*-Gravenhage: Staatsdrukkerij-en Uitgeverijbedrijf, 1958.) [77

U.S. Department of Commerce: Coast and Geodetic Survey. Technical Bulletin No. 4 (February 1959): Radio Telemetry Applied to Survey Problems. By Richard R. Ross. Pp. ii+7. (Washington, D.C.: Government Printing Office, 1959.) 15 cents. [77

Museum of Comparative Zoology at Harvard College. Breviora. No. 102 (April 9, 1959): A New Jamaican Galliwasp (Sauria, Anguidae). By Garth Underwood. Pp. 13. No. 103 (April 10, 1959): Two New Species of *Eleutherodactylus* from Puerto Rico. By Juan A. Rivero. Pp. 6. No. 104 (April 13, 1959): Studies on Fishes of the Family Ophidiidae. 3: A New Species of *Lepophidium* from Barbados. By C. Richard Robins. Pp. 7. No. 105 (April 14, 1959): *Bufo gundlachi*, a New Species of Cuban Toad. By Rodolfo Ruibal. Pp. 14. No. 106 (April 28, 1959): The Occipito-Vertebral Joint in the Burrowing Snakes of the Family Uropeltidae. By Ernest W. Williams. Pp. 10. No. 107 (May 6, 1959): A Revision of the Dacetine Ant Genus *Acanthognathus*. By William L. Brown, Jr. Pp. 13. No. 108 (May 7, 1959): Some New Species of Dacetine Ants. By William L. Brown, Jr. Pp. 11. No. 109 (May 8, 1959): On the Pineal Area and Adjacent Structures of the Brain of the Dipnoal Fish, *Protopterus annectens* (Owen). By Uno Holmgren. Pp. 7+2 plates. (Cambridge, Mass.: Museum of Comparative Zoology at Harvard College, 1959.) [77

Proceedings of the United States National Museum. Vol. 109, No. 3414: Flies of the Genus *Odinia* in the Western Hemisphere (Diptera: Odiniidae). By Curtis W. Sabrosky. Pp. 223–236+1 plate. (Washington, D.C.: Government Printing Office, 1959.) [77

Bulletin of the Museum of Comparative Zoology at Harvard College. Vol. 120, No. 3: The Rodents of the Deseadan Oligocene of Patagonia and the Beginnings of South American Rodent Evolution. By Albert E. Wood and Bryan Patterson. Pp. 419–428. Vol. 120, No. 4: The Types of Corbiculidae and Sphaeriidae (Mollusca: Pelecypoda) in the Museum of Comparative Zoology, and a Bio-Bibliographic Sketch of Temple Prime, an Early Specialist of the Group. By Richard I. Johnson. Pp. 429–480+8 plates. Vol. 120, No. 5: Studies on the Fauna of Melanesia. 5: The Tribe Odontomachini. By Edward O. Wilson. Pp. 481–510+2 plates. Vol. 121, No. 1: Description of the Skull of *Pomatodelphis inaequalis* Allen. By Remington Kellogg. Pp. 1–26+6 plates. Vol. 121, No. 2: Land and Freshwater Mollusks of Great and Little Inagua, Bahama Islands. By William J. Clench. Pp. 27–54+1 plate. (Cambridge, Mass.: Museum of Comparative Zoology at Harvard College, 1959.) [77

United Nations. Economic Developments in the Middle East, 1957–1958. (Supplement to World Economic Survey, 1958.) Pp. viii+104. (New York: United Nations; London: H.M. Stationery Office, 1959). 1.25 dollars; 9*s.*; 5 Swiss francs. [77

Rubber Research Institute of Malaya. Annual Report 1956. Pp. 79. (Kuala Lumpur: Rubber Research Institute of Malaya, 1959.) [77

Arctic Institute of North America. Technical Paper No. 3: The Vegetation of Northern Manitoba. 3: Studies in the Subarctic. By J. C. Ritchie. Pp. 56+8 plates. (Montreal: Arctic Institute of North America, 3485 University Street, 1959.) 2 dollars. [77

*Geological Survey of Uganda. Records of the Geological Survey of Uganda 1955–56.* Pp. ii+50+4 plates. 8*s.* 7/50. Report No. 1: The Geology of Southern Mengo. Explanation of Sheet $\frac{\text{North A—36}}{\text{U}}$ and part of sheet $\frac{\text{North A—36}}{\text{V}}$. By J. W. Pallister. With an Appendix on Petrology by Dr. C. G. B. Du Bois, and a section on the Soils of Buganda by Dr. E. M. Chenery. Pp. iii+124+5 maps. 8*s.* 50. (Entebbe: Government Printer, 1959.) [77

New Zealand Forest Service: Forest Research Institute. Technical Paper No. 24: The Physical and Mechanical Properties of New Zealand Grown Douglas Fir. By J. Maddern Harris and H. R. Orman. Pp. 88+5 plates. Technical Paper No. 26: The Drying of *Radiata* Pine Sawn Timber Pressure Treated with Water-Borne Preservatives. By J. A. Kininmonth. Pp. 32. (Wellington: Director of Forestry, 1958.) [77

Bulletin of the Florida State Museum, Biological Sciences. Vol. 4, No. 8: The Epaxial Musculature of *Siren*, *Amphiuma*, and *Necturus* (Amphibia). By Walter Auffenberg. Pp. 253–266. 25 cents. Vol. 4, No. 9: The Pleistocene Avifauna of Arredondo, Florida. By Pierce Brodkorb. Pp. 267–292. 35 cents. Vol. 4, No. 10: The Atlantic Loggerhead Sea Turtle, *Caretta caretta caretta* (Linnaeus) in America. By David K. Caldwell, Archie Carr, and others. Pp. 293–348. 70 cents. Vol. 4, No. 11: Pleistocene Birds from New Providence Island, Bahamas. By Pierce Brodkorb. Pp. 349–372. 35 cents. (Gainesville, Fla.: Florida State Museum, 1959.) [77

United States Department of Agriculture: Agricultural Research Service. Technical Bulletin No. 1198: A Review of Leafhoppers of the Genus *Dravidarcephala*. By David A. Young and Ralph H. Davidson. Pp. 32. (Washington, D.C.: Government Printing Office, 1959.) 15 cents. [77

*Editorial and Publishing Offices of "NATURE"*
MACMILLAN & CO., LTD.,
ST. MARTIN'S STREET, LONDON, W.C.2.
Telephone Number: Whitehall 8831. Telegrams: Phusis Lesquare London

Annual subscription £7/15/–, payable in advance, postage paid to any part of the world

*Advertisements only should be addressed to*
T. G. Scott & Son, Ltd., 1 Clement's Inn, London, W.C.2
Telephone Number: Holborn 4743

All rights reserved. Registered as a newspaper at the General Post Office

# LETTERS TO THE EDITORS

## ASTROPHYSICS

### The Upper Boundary of the Van Allen Radiation Belts.

THREE measurements have now been made of the intensity and extent of the Earth's upper radiation belt and of the primary cosmic-ray intensity beyond. These were made by: (1) the American space probe, *Pioneer III (December 6-7, 1958, ref. 1)*, (2) the Russian cosmic rocket, *Mechta* (January 2-4, 1959, ref. 2), and (3) the American space probe, *Pioneer IV* (March 3-6, 1959, ref. 3). Since the radiation belts vary with time, the three experiments give different results for the intensity of the belts, but the measurements of cosmic-ray intensity beyond the belts ought to be comparable. Nevertheless, preliminary results of the *Pioneer* tests seemed to indicate a difference in cosmic-ray intensity of almost a factor of 2. This communication discusses the resolution of this discrepancy and some new data bearing on the spatial extent of the trapped radiation.

On *Pioneer IV*, the counting-rate was observed to be a constant, 1·09 counts/sec., beyond a range of 91,000 km. (measured from the centre of the Earth). Since this rate persisted for 85·9 hr., during which the range of the probe increased by 564,000 km., it is clearly characteristic of the interplanetary particle flux.

*Pioneer III*, on the other hand, never reached a region where the counting-rate ceased to vary with altitude. Van Allen[1] suggested that the upper boundary of the radiation belt might reasonably be considered to be 64,000 km., since the rate of decrease of counting-rate with increasing range is very small above this altitude (see Fig. 1). Actually, however, when the Goldstone tracking station lost radio contact with the probe at a range of 107,500 km., the counting-rate had dropped to only 2·25 counts/sec. and appeared to be still dropping at approximately 0·01 count/sec. per 1,000 km.

It was reasonable to assume, as Van Allen did, that the 2·25-counts/sec. rate was near the asymptotic value, and that the trapped radiation contributed negligibly to the counting-rate. It will be shown here that these assumptions are not valid, that the counting-rate actually dropped considerably lower, and that therefore the puzzling descrepancy between the measurements of *Pioneers III* and *IV* probably does not indicate a change in the primary cosmic-ray flux, as has been suggested[3].

The Goldstone tracking station lost contact with *Pioneer III* at 22·46.30 U.T., about 16·6 hr. after launch and 2·5 hr. before it reached apogee. It was expected to rise over the horizon at Puerto Rico about 13 hr. later at a range of 87,000 km., and the tracking station there attempted to re-acquire its signal. At first, only occasional momentary phase lock-ons were obtained, but their duration and frequency increased steadily, and after 15·20 U.T., the telemetry data were, to use Van Allen's phrase, "solid". They continued to be solid until the probe set over Puerto Rico's eastern horizon at 19.29 U.T., about 3,300 km. over the central Atlantic. During solid telemetry, the counting-rate rose rapidly, as shown by the solid curve in Fig. 1, from about 3·3 counts/sec. to very high rates in the radiation belts.

Even without reading the doubtful region of the telemetry record, it can be shown as follows that the rate of 2·25 counts/sec. cannot correspond to the cosmic-ray counting-rate alone.

The 17-stage scaling circuit was provided with three output taps so that distinctive signals (which we call 'down flips') occur at intervals of $2^9$, $2^{13}$, and $2^{17}$ counts, and 'up flips' halfway between. At low counting-rates, all six different kinds of scaler flips are distinguishable. Thus, between the last stage-17 up flip on the Goldstone record, at 21.55.57 U.T., and the first one on the Puerto Rico re-entry record, at 16.17.24 U.T. the next day, the counter must have recorded $2^{17}\ n = 131{,}072\ n$ counts (where $n$ is an integer). Between these times, 35 stage-9 cycles, corresponding to 17,820 counts, appear on the solid-telemetry record, and hence we can calculate the number of counts which the probe detected when we were not watching it. Thus

Interval of missing telemetry: 22.44.32 to 15.21.36
Time interval: 61,624 sec.
Counts: $131{,}072\ n - 17{,}820$
Mean counting-rate (counts/sec.): $1{\cdot}836 + 2{\cdot}127\ (n-1)$

Thus, the mean counting-rate in the interval of missing telemetry was either (1) 1·836 counts/sec. if $n = 1$, in which case the minimum counting-rate was considerably lower, or (2) 3·963 or 6·090 counts/sec. or some higher value if $n > 1$, in which case the probe must have run into a region of radiation of high intensity near its apogee and would probably never have recorded cosmic-ray background.

Further evidence is obtained by a re-examination of the telemetered data. The telemetry was recorded on magnetic tape. By playing the Puerto Rico tape back into a pen recorder using a compressed time-scale and an expanded frequency-scale, it is possible to obtain a graph which cannot be read accurately but on which the pattern of scaler flips is clearly discernible back to about 13.00 U.T., fairly readable back to 12·30 U.T., and possibly distinguishable as early as 12·00 U.T. With the time-compressed record as a guide, it is possible to locate some of the scaler flips on the original record to within a few seconds and to obtain reliable data well before 15·20 U.T.

In Fig. 1, the solid curve shows the counting-rate where it is well known from solid telemetry, and the experimental points are our best estimate of the data in the region where phase lock is intermittent. The data shown as circles were obtained by reading flip times from the original record and are essentially as reliable as the 'solid' data. Each point represents 768 counts, and times can be read to a few seconds. The data marked as triangles were obtained by reading the played-back time-compressed record with an accuracy of about a minute; their reliability is increasingly doubtful at earlier times. Each point represents 512 counts. It appears clear that the counting-rate dropped to as low as approximately 1·4 counts/sec., and that even this value probably

does not correspond to cosmic-ray background, since it was observed at a range of only 65,000 km., below a region of higher intensity.

The two dashed curves in Fig. 1 show the implications of assuming different values of $n$. The lower one corresponds to $n = 1$ and the upper to $n = 2$. Their exact shape is arbitrary, but they are adjusted so that the area under each corresponds to the proper number of counts in the interval of absent or intermittent telemetry, while still making the curves as simple as possible. It is, of course, possible that the curves should show additional peaks and valleys, such as were seen by *Pioneer IV*.

The lack of symmetry about apogee of the high-altitude portion of the counting-rate curve could indicate a spatial or a temporal variation of the radiation itself or could result from the directional response of the counter. The first explanation seems most reasonable, since the probe was considerably farther south on descent than on ascent. An altitude-versus-latitude plot of the trajectory is given in ref. 1,

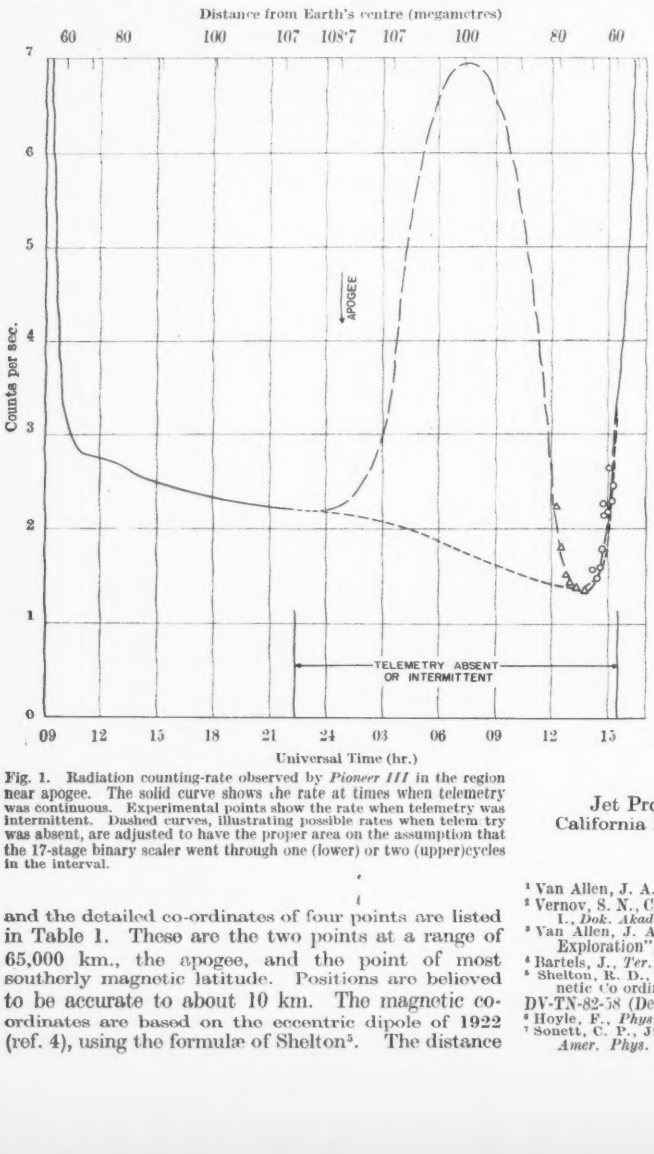



Fig. 1. Radiation counting-rate observed by *Pioneer III* in the region near apogee. The solid curve shows the rate at times when telemetry was continuous. Experimental points show the rate when telemetry was intermittent. Dashed curves, illustrating possible rates when telemetry was absent, are adjusted to have the proper area on the assumption that the 17-stage binary scaler went through one (lower) or two (upper) cycles in the interval.

and the detailed co-ordinates of four points are listed in Table 1. These are the two points at a range of 65,000 km., the apogee, and the point of most southerly magnetic latitude. Positions are believed to be accurate to about 10 km. The magnetic co-ordinates are based on the eccentric dipole of 1922 (ref. 4), using the formulæ of Shelton[5]. The distance

Table 1. Calculated Position Co-ordinates of *Pioneer III* at Selected Times

| Position co-ordinates | Universal time | | | |
|---|---|---|---|---|
| | Dec. 6, 10.48 | Dec. 7, 00.45 | Dec. 7, 03.45 | Dec. 7, 14.43 |
| Range (km.) | 65,000 | 108,700 | 106,950 | 65,000 |
| Longitude (deg.) | 26·5 W. | 146·5 E. | 105·1 E. | 38·4 W. |
| Latitude (deg.) | 15·3 S. | 23·7 S. | 24·7 S. | 28·4 S. |
| Right Ascension (deg.) | 210·1 | 233·0 | 236·8 | 258·3 |
| Declination (deg.) | −15·3 | −23·7 | −24·7 | −28·4 |
| Magnetic range (km.) | 64,670 | 108,990 | 107,100 | 64,720 |
| Magnetic latitude (deg.) | 6·6 S. | 32·8 S. | 36·2 S. | 18·4 S. |

between the two points at 65,000-km. range is 43·3° or about 51,000 km.

Vernov *et al.*[2] state that the upper boundary of the radiation belt as seen by *Mechta* was at a range of 55,000 km. near the magnetic equator. *Pioneer IV* detected numerous narrow peaks of radiation intensity between 47,000 and 91,000 km., but the rapid drop in intensity beyond the main peak of the radiation belt appears to end at about 60,000 km. The curve of Fig. 1 for *Pioneer III* is fairly accurately symmetrical about apogee between 40,000 and 60,000 km. These three facts strongly point to the region of 9–10 Earth radii as being the upper boundary of the radiation belts. This accords well with the theoretical considerations of Hoyle[6] and the magnetometer measurements of *Pioneer I* (ref. 7), which place the upper boundary of the Earth's magnetic dipole field in the same region. Probably the radiation above cosmic-ray background at greater ranges than this is trapped on magnetic field lines which are still attached to the Sun. The amount of such radiation observed by the three probes which have penetrated the region correlates well with the amount of solar activity in the few days preceding their launching.

This communication presents the results of one phase of research carried out at the Jet Propulsion Laboratory, California Institute of Technology, under Contract No. *NASw*-6, sponsored by the National Aeronautics and Space Administration.

Conway W. Snyder

Jet Propulsion Laboratory,
California Institute of Technology,
Pasadena.

[1] Van Allen, J. A., and Frank, L. A., *Nature*, **183**, 430 (1959).
[2] Vernov, S. N., Chudakov, A. Ye, Vakulov, P. V., and Lofachev, Yu. I., *Dok. Akad. Nauk. S.S.S.R.*, **125**, 304 (1959).
[3] Van Allen, J. A., reported at symposium on "Problems of Space Exploration", Nat. Acad. Sci., Washington, April 29, 1959.
[4] Bartels, J., *Ter. Mag. and Atm. Elect.*, **41**, 225 (1936).
[5] Shelton, R. D., "The Transformation from Geographic to Geomagnetic Co ordinates", Army Ballistic Missile Agency Report No. DV-TN-82-58 (Dec. 18, 1958).
[6] Hoyle, F., *Phys. Rev.*, **104**, 269 (1956).
[7] Sonett, C. P., Judge, D. L., Kelso, J. M., and Sims, A. R., *Bull. Amer. Phys. Soc.*, **2**, 222 (1959).

## Formation of Stellar Assciations from Galactic Gas

Galactic gas is present in three types of regions. According to Schlüter[1], these are: (1) cold dense HI clouds of temperature of the order of 100° K. and density of 10 atoms/cm.³, occupying about 5 per cent of space; (2) these are surrounded by a hot transparent continuous gas at 10,000° K., density 0·1 atom/cm.³ and pressure $10^{-13}$ dyne/cm.²; (3) in addition, about 0·5 per cent of space contains hot dense rapidly expanding H II regions, heated by nuclear energy derived from embedded *O* or *B* type stars supernovæ and novæ. The temperature of 10,000° K. of the main continuous component is probably maintained by turbulent and viscous dissipation of rotational galactic shear, which can supply $10^{-25}$ erg/cm.³ sec. for $6 \times 10^9$ years, and by absorption of stellar radiation. The energy sink is provided by radiation into intergalactic space. The H I regions must have a comparatively short life of some $10^7$ years and must be replenished. Steady population of all ages must develop, in which condensations are born from the hot continuous component by Zanstra-type instability based on strong dependence of cooling of ionized gas on density. Denser eddies of ionized gas continue losing internal pressure by excessive radiative cooling and collapse from the excess pressure of the surround hot gas. After recombination, cooling-rate slows down, but shear energy supply disappears. An ideal spherical region would deform under galactic shear first into an ellipsoid, which would then continue to collapse into an elongated disk of very small thickness. Collisions of H I regions and turbulence of the continuous component combined with galactic shear would tend to disperse such sheets and return their contents to the hot gas, closing thus their cycle of evolution.

The hot component is turbulent at Mach number or order 0·5 as a consequence of large Reynolds number. However, the galaxy is stable against large-scale turbulence. There must therefore be a maximum size of an eddy, which cannot exceed 3,000 parsecs, as then the Rayleigh stability criterion $d(vr)/dr > 0$ begins to work. This probably accounts for the observed peculiar velocities, sizes and masses of the HI regions, whereas the large-scale appearance of spiral arms is laminar.

The life-cycle of the larger H I regions may be modified by gravitational self-attraction. Gravitational effects in thin sheets are much stronger than in three-dimensional regions of the same total mass and lateral extension. Forces similar to surface tension of liquids appear, although gravitation is a long-range force, whereas in liquids only short-range forces contribute to the surface tension. These tend to keep the sheets infinitely thin and planar. Local condensations of the type suggested by Jeans may form, which may account for some stars and clusters, especially at the galactic rim. However, a more efficient process has been found, similar to bursting of a membrane under surface tension. When a thin sheet of self-gravitating matter is pierced by a hole, for example, by an accidental presence of a hot star, the hole will begin to expand by the appearance of radial forces at the rim. An expanding ring will form, which will increase its mass as it sweeps up the material of the sheet. Instability may arise along the ring, leading to the formation of a chain of stars, located on an expanding circle.

Galactic shear and magnetic fields may modify this process, causing anisotropy of expansion. Magnetic lines of force may become drawn along part of the ring and the resulting stars would assume magnetic dipoles directed along the ring.

Search of stellar photographs reveals many elliptical chains of stars, which may be identified with stellar associations. When searching photographs, very elongated ellipses become more clearly visible to the eye when the photographs are viewed obliquely so that the ellipses become foreshortened into circles.

Some star chains so observed appear to be double, with a dark lane running in between the two parallel ellipses. This phenomenon can readily be explained by the present theory. If the ring is fed by a sufficiently planar sheet, two vortices of opposite circulation may form, one above and one below the sheet. They will hydrodynamically repel each other. The rings may disintegrate into two parallel chains of stars, spinning with their axes parallel to the local tangents to the rings. Corresponding pairs of stars above and below the sheet would rotate in opposite directions. Trapped magnetic fields will generally be parallel to the rotation axes. In the outermost layers of the protostars, angular momentum would be available for planet formation, whereas inner portions of stars would tend to rotate but slowly. Planetary systems should be common.

Rough order-of-magnitude calculation suggests that a ring 10 parsecs in diameter formed in a sheet 0·01 parsec thick and of surface density $3 \times 10^{-2}$ gm./cm.² would have radial expansion velocity 5 km./sec. and mass of 10,000 solar masses. Much thinner sheets would produce comparable expansion velocities at lower surface densities and masses.

I wish to express my thanks to Prof. G. Gamow for helpful discussion. This work has been done when under appointment as a visiting professor in the Chemistry Department, University of Colorado.

Vladimir Vand*

Chemistry Department,
University of Colorado,
Boulder, Colorado.
July 10.

* Present address: Crystal Research Laboratory, Physics Department, Pennsylvania State University, University Park, Pennsylvania.
[1] Schlüter, Internat. Astro. Union Symposium No. 2, p. 144 (North-Holland Pub. Co., Amsterdam, 1955).

## An Error in the Determination of $\Delta T$ from the Lunar Ephemeris and the Frequency of Caesium in Terms of U.T. + $\Delta T$

The ellipticity of the Earth causes perturbations of the elements of the Moon's orbit with a period of 18·6 years. The value of the ellipticity used in Brown's "Tables of the Motion of the Moon" and in the "Improved Lunar Ephemeris" is 1/294. As has been pointed out elsewhere[1], the correction to be applied to the tabular longitude of the Moon in order to reduce it to ellipticity 1/297 is equal to $-0{\cdot}149''$ sin Ω, where Ω is the longitude of the Moon's node. This is equivalent to a correction of + 0·271 s. sin Ω to $\Delta T$ as defined by the International Astronomical Union[2]. Recent observations of artificial Earth satellites indicate that the true value of the ellipticity may be nearer 1/298 (ref. 3), in which case the correction to be applied to $\Delta T$ is + 0·36 s. sin Ω.

A value for the frequency of cæsium in terms of U.T. + $\Delta T$ has been published by Markowitz and

others[4] based on the change of $\Delta T$ in the interval 1955·50–1958·25. The corrections which should be applied to $\Delta T$ at these epochs, on the assumption that the true value of the ellipticity is 1/298, are −0·36 s. and −0·19 s., respectively, and the corresponding correction to this particular determination of the frequency is −18 c./s.

More generally, if $e$ is the true value of the ellipticity, then the correction to be applied to an observed frequency of cæsium in terms of U.T. + $\Delta T$, where $\Delta T$ has been determined from the lunar ephemeris, is $+ 8{\cdot}8\ (e^{-1} - 294) \cos \Omega$ c./s.

This communication is published by permission of H.M. Astronomer Royal.

C. A. Murray

Royal Greenwich Observatory.
Herstmonceux Castle,
Hailsham, Sussex.

[1] Murray, C. A., *Mon. Not. Roy. Astron. Soc.*, **116**, 477 (1956).
[2] *Trans. Int. Astron. Union*, **8**, 66 (1954).
[3] Cook, A. H., *Geophys. J.*, **1**, 341 (1958).
[4] Markowitz, W., Hall, R. Glenn, Essen, L., and Perry, J. V. L., *Phys. Rev. Letters*, **1**, 105 (1958).

## GEOPHYSICS

### Geophysical Effects of High-Altitude Nuclear Explosions

Recent observations of geophysical effects of high-altitude nuclear explosion[1] have indicated that such blasts give rise to signals similar to solar flares when recorded in the 27 kc./s. range. A re-examination of the 27 kc./s. record of August 12, 1958, obtained in Pittsburgh, Pa., shows a striking similarity to the integrated atmospherics obtained in Japan, but delayed by about 1 hr.

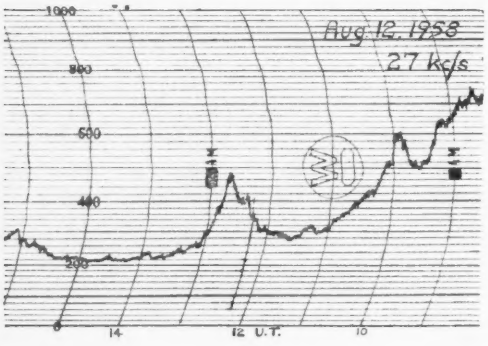



Fig. 1

The accompanying graph (Fig. 1) shows the enhancement between 12 and 13 hr. U.T. The line at 12.15 (8.15 E.D.T.) is a time check mark. Local sunrise was at 10 U.T. and is shown by the characteristic sunrise hump. This sunrise effect is present on all records previous to and following August 12. The local weather report for August 12 indicates clear skies at sunrise, followed by fog later in the morning and thunderstorms in the late afternoon. There was no major solar activity at the time of enhancement.

While these results are not entirely unambiguous, they may add interesting speculation on the detectability of high-altitude nuclear blasts.

W. A. Feibelman

1063 Findley Dr.,
Pittsburg 21, Pa.
July 2.

[1] Obayashi Coroniti and Pierce, *Nature*, **183**, 1476 (1959).

## PHYSICS

### Quadrupole Anti-Shielding Factor in Copper

Recent measurements of the nuclear magnetic resonance absorption line in heavily deformed copper sheet[1] showed that, although the plastic deformation caused some reduction in the intensity of the line, this reduction was only one-third of that predicted by Bloembergen[2] on the basis of nuclear quadrupole interactions; furthermore, there was no apparent broadening of the line.

Assuming a value of $\lambda = 60$ for the quadrupole anti-shielding factor in the copper lattice (see below), Bloembergen showed by an approximate calculation that a dislocation density of $3 \times 10^8$ lines/cm.$^2$ would be sufficient to render unobservable the satellite components of the resonance line, and thus cause a reduction of 60 per cent in the intensity of the observed line. In the specimens of rolled sheet referred to above, which had undergone 25 per cent–90 per cent reduction in thickness by rolling, the dislocation density was probably[3] of the order of $10^{11}$ lines/cm.$^2$ and the reduction in intensity compared with the annealed material was only 20 per cent; it therefore appears that the estimate of $\lambda = 60$, which is deduced indirectly from measurements on copper-zinc alloys, may be too high.

In the present investigation a series of experiments has been performed with the object of determining $\lambda$ directly from measurements of the broadening of the resonance line in specimens of copper subjected to elastic strain. The maximum strains available are of the same order of magnitude as those to be expected, on the basis of a simple model, in the main part of the strain field due to a random array of dislocations with a density of $10^{10}$ lines/cm.$^2$

The specimens were made from the same sample of electrolytic copper that was used in the earlier experiments on plastic deformation; a preliminary experiment on annealed filings showed that the intensity of the resonance line was the same as that from an annealed sample of spectroscopically pure copper. Each specimen was a strip $2{\cdot}0 \times 0{\cdot}6 \times 0{\cdot}0045$ cm. which had been annealed for $2\frac{1}{2}$ minutes at a temperature of 300° after 97 per cent reduction in thickness by rolling. Metallographic examination revealed that the material had fully recrystallized and had a grain size of 5–10 μ. The strip was in the shape of a spiral of $1\frac{1}{2}$ turns with an air space of 0·1 cm. between adjacent surfaces; this was mounted in a special holder in which the spiral could be 'wound up' like a clock spring while remaining in the specimen coil of the spectrometer. It was found that after the centre had been rotated in either sense through an angle of 45° there was less than 5° change in the equilibrium position, from which it was assumed that this deformation was predominantly within the elastic range; it corresponds to a maximum strain of approximately $9 \times 10^{-4}$.

The nuclear magnetic resonance absorption line was observed by means of a Colpitts marginal oscillator operating at a frequency of 5·5 Mc./s., with the specimen in the equilibrium position and in the deformed positions. About 50 lines were recorded from 5 specimens. The result was that no difference could be detected, either in width or intensity, between the lines obtained in the two states. The signal-noise ratio was reasonably high (about 5:1 with an inte-

grating time of 5 sec.) and, in view of the fact that a homogeneous type of broadening would be expected, it was estimated that a change of 20 per cent in the mean square line width would have been easily observable. Since the normal mean square line width is about (3 kc./s.)[2], the following upper limit can be placed on the contribution of quadrupolar strain-broadening to the width of the satellite components:

$$\overline{\Delta\nu_Q{}^2} < (1{\cdot}6\ \text{kc./s.})^2 \qquad (1)$$

The quadrupolar perturbation of the nuclear magnetic resonance at a given site depends on the electric field gradient grad grad $\varphi$, which is connected with the strain at the site by a fourth-order tensor which may be written, by the use of the Voigt notation, in terms of components $F_{ij}$. An estimate $F'_{ij}$ of these components may be obtained if it is assumed that the field gradient is due to single electronic charges $e$ located at the lattice sites in a uniformly deformed crystal; for the twelve nearest neighbours in a face-centred cubic lattice with nearest-neighbour distance $a$, these components are

$$F'_{11} = -2F'_{12} = 6ea^{-3}, \quad F'_{44} = F'_{12} \qquad (2)$$

and the anti-shielding factor $\lambda$ may be defined by the equation

$$\lambda = F_{11}/F'_{11} \qquad (3)$$

It must be emphasized that $\lambda$ is not, in general, the same as the Sternheimer factor $(1 + \gamma_\infty)$.[4]

In first-order quadrupolar interaction the only operative component of grad grad $\varphi$ is $\partial^2\varphi/\partial z^2$ where $z$ is the direction of the applied magnetic field. The effect of a simple compressional strain $\varepsilon$ in polycrystalline material may be estimated by calculating $\partial^2\varphi/\partial z^2$, in terms of the components of $F$, as a function of crystal orientation and averaging over all possible orientations to find the mean square value: the result (assuming that $F_{44} = F_{12}$) is

$$\overline{\left(\frac{\partial^2\varphi}{\partial z^2}\right)^2} = \varepsilon^2 F^2_{11} \cdot \frac{1}{84}(3 + \cos^2 A)^2 \qquad (4)$$

where $A$ is the angle between the strain and the $z$ axis.

An upper limit can now be set on the value of $\lambda$. Using equations 1–4 in conjunction with the well-known formulae for first-order quadrupole interaction[5], and averaging over all values of $A$ and $\varepsilon$, the result is $\lambda < 7$.

In this calculation, the usual assumption has been made that the satellite components make their full contribution to the nuclear magnetic resonance in pure annealed copper. However, the possibility cannot be excluded that $\lambda$ has a very high value and that the satellite components do not contribute even in the annealed state.

E. A. Faulkner

Division of Tribophysics,
Commonwealth Scientific and
Industrial Research Organization
University of Melbourne.

[1] Faulkner, E. A., *Nature*, **183**, 1043 (1959).
[2] Bloembergen, N., Bristol Conference on Defects in Crystalline Solids, 1 (London: Phys. Soc., 1955).
[3] Clarebrough, L. M., Hargreaves, M. E., and West, G. W., *Acta Met.*, **5**, 738 (1957).
[4] Foley, H. M., Sternheimer, R. M., and Tycko, D., *Phys. Rev.*, **93**, 734 (1954).
[5] Pound R. V., *Phys. Rev.*, **79** 685 (1950).

## Solid State Rheological Study of the Mechanism of Paraffin-Detergent Interactions in Aqueous Systems

The generation of new surfaces in a solid by rheological means affords the opportunity of studying the behaviour of these clean surfaces in respect to specific surface reactions. Thus a sensitive stress-strain record of the slow-plastic deformation of a solid is somewhat akin to the Heyrovsky dropping mercury polarograph in the respect of fresh surfaces being available for analytical (chemical, physical or electrochemical) studies. As a part of a fundamental 'Instron' precision tension apparatus study of first- and second-order transitions in some solid paraffins, we made a number of stress-strain tests of the mechanism of paraffin-detergent interactions in aqueous solutions. We wished to see whether the new surfaces produced by a slow (0·50 in./min.) plastic extension would interact to a measurable degree with two detergent solutions. Four representative specimens were tested in each of three environments: distilled water; a 0·4 per cent solution of technical grade sodium lauryl sulphate in distilled water; and a 0·4 per cent solution of technical grade sodium dodecyl benzene sulphonate in hard water. (The approximate composition of this Ponca City hard water was: calcium, 110; magnesium, 34; sodium, 180; potassium, 4; chloride, 284; sulphate, 167; bicarbonate, 329 p.p.m., the total solids being 888 p.p.m.) The hard water solution was used according to a suggestion of Dr. W. H. Harwood of our Research Department, who indicated (this was easily proved by simple beaker dipping tests) that a greater paraffin wetting action might be expected in waters containing the divalent ions of calcium and magnesium, these forming the corresponding detergent salts of decreased solubilities. The tensile specimens were prepared from a thin paraffin sheet formed by pouring liquid paraffin upon a clean, hot water surface.

There were found pronounced effects upon both the surface of the paraffin wax tensile specimens and upon their elongations. With the detergent molecules present there were myriads of surface cracks which had a tendency to be normal to the surface and normal to the longitudinal axis of the specimen. We interpreted and expected that the preferred orientation of the paraffin crystals controlled this particular rheological response. Table 1 illustrates the results

Table 1. Elongation Measures of Detergent-Paraffin Interactions (using an 'Instron' precision tension test instrument)
Test Results

| Condition | Elongation, Average of Four Specimens Each | Elongation Ratio (%) |
|---|---|---|
| 1. Distilled water elongation | 1·777 in. | 100 |
| 2. With 0·4 per cent solution of sodium lauryl sulphate | 1·210 in. | 68·2 |
| *3. With 0·4 per cent solution (hard water) of sodium dodecyl benzene sulphonate | 0·936 in. (3 specimens) | 52·8 |

* The surface checking effects of Run No. 3 were sharper and deeper than with No. 2. Here also were the best geometric indications upon the surfaces of the tensile specimens of the direct relation of the paraffin structural orientation to the crack form and orientations.
Specimens: 6½ in. long; $\frac{5}{8}$ in. thick; 1 in. wide at shoulder; ½ in. wide at waist; 2 in. shoulder-to-shoulder length.
Tensile strength: 122 lb. per sq. in. (not seriously affected by detergent solution contacts), the average of eight specimens tested at 22° C.

obtained. No special strength effects were noted. We interpret this to mean that strength is primarily a volume property, whereas elongation is a strongly surface-limited property. It will be noted that the detergent-paraffin system of the sodium dodecylben-

zene sulphonate in hard water was the most damaging to the elongation. Four specimens were employed for each data point. One specimen was tested in sodium lauryl sulphate at a concentration of 0·01 per cent, but the elongation thereof was such as to fall within the scatter range of the distilled water data.

We conclude that the wetting effects caused by the detergent molecules cause a serious elongation reduction, that the mechanism of this is probably due to the no-repair possibility of extremely fine surface cracks in the paraffin by the wetting action of the detergent solution. If the second point be true, then it would appear that there are many surface cracks or microfissures which are at least partly healed during tensile flows under ordinary circumstances. From the data it also can be seen how important the testing environment is. To the extent of our data (four separate runs 0 per cent, 0·01 per cent and 0·4 per cent sodium lauryl sulphate in distilled water and 0·4 per cent sodium dodecyl benzene sulphonate in hard water), the elongation impairment is in a direct relation to the observed wetting action.

These data have a multiplicity of possibilities, as they not only show the rheological consequences of the paraffin-detergent surface interactions but they also indicate how paraffinic or other solids could be studied for use in extraction or for chromatographic purposes. Perhaps the data afford the explanation possibility of why it is that the commercial detergent product, as 'Tide', happens to be a good chromatographic column material for various hydrocarbon gases—herein again is the detergent-paraffin system but in an inversed relation as to the solid and the dilute (gaseous) states. The paraffin-detergent interactions also have at least two significant industrial aspects. One is that some of the solid long-chain hydrocarbons are in widespread use for milk cartons, wax paper and various food packaging applications. The other is in the field of plastics testing: there exists a standard stress-corrosion cracking test of polyethylenes in a certain nonionic detergent solution. This elongation test may be a supplement to or a substitution for the above stress corrosion cracking system. Finally, whereas it had been shown that aqueous proteins and milks of various fat contents were capable of eluting certain complex hydrocarbons from wax surfaces, we are unaware of studies of the reverse system[1].

We are indebted to Dr. William H. Harwood for his comments and interest in these studies.

F. J. Radd
L. H. Crowder

Continental Oil Co., P.O. Drawer 1267,
Ponca City, Oklahoma.
July 9.

[1] H. L. Falk, P. Kotin, A. Miller, *Nature*, **183**, 1184 (1959).

## Action of Mixed Solvents on Wool

During the course of another investigation[1] it was observed that the resistance of wool fibres to extension in water is greater than in butanol saturated with water. This surprising result, which has an important bearing on methods of determining the accessibility of wool to different reagents[2], led to a study of the behaviour of wool fibres in mixtures of other primary alcohols and water, as well as in mixed solvents generally. Among the more interesting results so far obtained are those for *n*-propanol and water. After being calibrated by 30 per cent extension in distilled water at 22·2° C., merino wool fibres (5-cm. lengths) were released and allowed to stand in distilled water for 24 hr., before transference to the propanol–water mixture for 18 hr., followed by re-extension in the mixture at 22·2° C. The percentage change in resistance to 30 per cent extension (C.R.E.) was calculated from the two load/extension curves for each fibre, and each of the results in Table 1 is the average of the values for 10 fibres.

Table 1.

| Propanol in mixture (per cent w/w) | C.R.E. (per cent) | Propanol in mixture (per cent w/w) | C.R.E. (per cent) |
|---|---|---|---|
| 0 | −1·5 | 50 | −10·9 |
| 10 | −5·5 | 60 | −7·6 |
| 20 | −8·7 | 70 | −5·7 |
| 30 | −8·8 | 80 | +2·5 |
| 40 | −10·7 | 90 | +52·2 |

Maximum weakening is obtained with the mixture containing 45 per cent (w/w) propanol, and in this mixture the diameter of Lincoln wool fibres was found to be 3·4 per cent greater than in water. The weakening is thus accompanied by swelling, which is greater than that of wool fibres in hydrochloric acid at *p*H 1 (ref. 3), and it seems probable that this is one of the causes of the success of solvent-assisted dyeing processes[4].

The form of the curve showing the C.R.E. as a function of the propanol content of the mixture is similar to that of corresponding curves for the turbidity temperature of solutions of zein and gliadin[5]. In addition, there are many similarities between the action of aqueous solutions of different primary alcohols in modifying the elastic properties of wool and in dissolving zein. It seems probable, therefore, that amide and inert side-chains of keratin are grouped in such a way that some sections of the chain molecules resemble those of zein, and that cohesion between such sections is weakened by the same solvent action which leads to the ready dissolution of zein in aqueous propanol.

J. C. Atkinson
A. Filson
J. B. Speakman

Department of Textile Industries,
University of Leeds.
May 11.

[1] Filson, A., and Speakman, J. B., *J. Soc. Dyers and Colourists*, **74**, 762 (1958).
[2] Speakman, J. B., *Proc. Roy. Soc.*, A, **132**, 167 (1931).
[3] Speakman, J. B., and Stott, E., *Trans. Farad. Soc.*, **30**, 539 (1934).
[4] Peters, L., and Stevens, C. B., *J. Soc. Dyers and Colourists*, **72**, 100 (1956).
[5] Dill, D. B., *J. Biol. Chem.*, **72**, 239 (1927). Manley, R. H., and Evans, C. D., *Indust. Eng. Chem.*, **35**, 661 (1943).

## Polynomial Representation of Thermodynamic Tables

In a recent communication Berry, Black and Enderby[1] describe an interesting use of the Tchebichef polynomials to represent the thermodynamic steam tables of Keenan and Keyes. This was a preliminary description which will presumably be followed by more details and a tabulation of the actual expansion coefficients. However, based on this initial communication, it is apparent that these authors have done a particularly elegant job of reducing a lengthy tabulation to a relatively small number of expansion coefficients. It is the purpose of this communication to point out the possibility of even further reduction in the number of coefficients required to represent the thermodynamic data[2].

For the gas phase, the tables of Keenan and Keyes actually present values of specific volume, specific enthalpy and specific entropy, with temperature and pressure as the independent variables. Berry *et al.* actually regarded the curve fitting as three separate problems: volume, enthalpy and entropy were each represented by orthogonal polynomial expansions in temperature and pressure. This is somewhat redundant thermodynamically. The expansion of volume as a function of temperature and pressure plus an expansion of the isobaric heat capacity as a function only of temperature at zero pressure would provide the necessary and sufficient information to compute explicitly values of enthalpy, entropy and other thermodynamic quantities.

These thermodynamic computations require differentiations and integrations of the volumetric and heat capacity data. One advantage of the Tchebichef polynomials is the availability of a set of relations which reduce integration and differentiation operations to simple algebraic operations. Lanczos[3] describes the integration identity. A differentiation identity, which is perhaps less known, is as follows:

$$Tn^{1}(x) = \frac{n}{2\,(1-x^{2})}\left[Tn_{-1}(x) - Tn_{+1}(x)\right]$$

Berry *et al.* reverted their Tchebichef polynomial expansion to a conventional integer power polynomial expansion. There may be substantial advantage in using the equation of state directly in the Tchebichef form. First, there exists a recursion formula which makes it feasible to generate values of the Tchebichef polynomials very rapidly, especially if the computations are being performed with a digital computer. Secondly, the availability of the differentiation and integration formulæ mentioned above, make it possible to obtain values of all thermodynamic quantities by simple matrix operations on the volumetric and heat capacity expansions. Thirdly, in the Tchebichef form the expansion coefficients are independent entities, which makes it possible to truncate the expansion arbitrarily if a lower-order approximation is required; this is in contrast to the power polynomial coefficients, which form a decidedly interdependent set for which truncation is disastrous.

A fuller description[4] of the use of orthogonal polynomials for representation of thermodynamic tables will be available shortly.

C. J. Pings

Department of Chemistry and
Chemical Engineering,
Stanford University,
Stanford, California.

[1] Berry, W. G., Black, G., and Enderby, J. A., *Nature*, 183, 598 (1959)
[2] Pings, C. J., and Sage, B. H., *Indust. Eng. Chem.*, 49, 1315, 1321 (1957).
[3] Lanczos, C., "Tables of Chebyshev Polynomials", Applied Mat. Series, No. 9 (Nat. Bur Stand., 1952).
[4] Opfell, J. B., Pings, C. J., and Sage, B. H., "Equations of State for Hydrocarbons" (American Petroleum Institute, New York, 1959).

## RADIOCHEMISTRY

### Thermal Decomposition of Irradiated Lead Oxalate

In a previous communication[1] it was suggested that the thermal decomposition of a solid would be affected by pre-irradiation if the decomposition of the unirradiated substance proceeds by a branching-chain mechanism, and if a simple metal cation is present. Lead oxalate satisfies these requirements[2], and we report here some preliminary results on the effects of pre-irradiation by gamma-rays on the subsequent thermal decomposition of this substance in the range 300–325° C.

The study of pre-irradiation effects necessitates a high degree of reproducibility of the decomposition of the irradiated and unirradiated material. The decompositions of the permanganates so far studied, and silver oxide, satisfied this requirement. However, when using lead oxalate prepared by the method formerly used[2] the decompositions were not sufficiently reproducible. This difficulty was overcome by precipitating the lead oxalate from $N/600$ sodium oxalate by the very slow addition of $N/5$ lead nitrate. The specimen was crystalline with particles approximately $4{\cdot}2 \times 10^{-3}$ cm. in diameter. The induction period prior to the main acceleration of the decomposition reaction was preceded by the rapid evolution of gas liberated during the decomposition of a surface layer of the oxalate. This conclusion was supported by the fact that immediately after this rapid reaction the particles were visibly coated with product. Pre-irradiations were carried out at room temperature in the spent fuel irradiation facility at Harwell. The γ-ray dose-rate was $4 \times 10^{6}$ rads hr.$^{-1}$. The effects of pre-irradiation doses of 70 Mrad and 250 Mrad on the decomposition are shown in Fig. 1. In all cases, for a constant mass of irradiated and unirradiated lead oxalate, the final gas pressure was the same within experimental error, showing that no measurable decomposition takes place during irradiation.

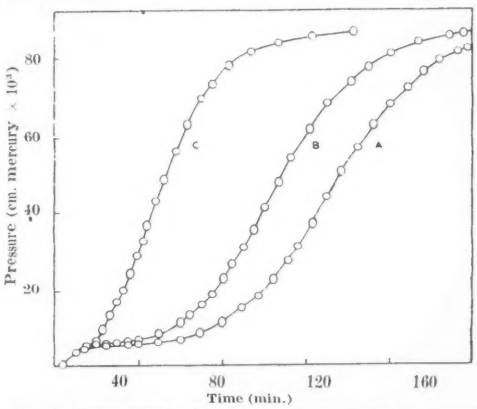
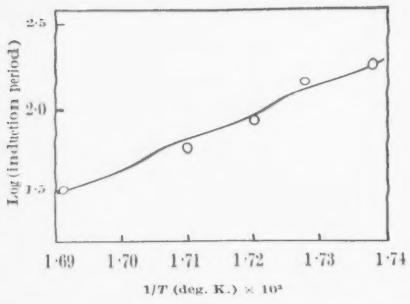



Fig. 1. Curves showing the effects of pre-irradiation by gamma-rays on the decomposition at 320° C. *A*, Unirradiated; *B*, 70 Mrad; *C*, 250 Mrad

The effects of pre-irradiation are similar to those found for potassium permanganate[3] and silver permanganate[4] in that the induction period is progressively shortened with an increasing dose of gamma-rays, and the acceleration of the reaction is increased. In the studies of potassium and silver permanganates it was suggested that the dependence of the length of the induction period on temperature, for a fixed irradiation dose, could be used to obtain values of the activation energy for the migration of point defects. The plot of $\log_{10}$ (induction period) against $1/T$ (deg. K.) for a gamma-ray dose of 70 70 Mrad on lead oxalate is shown in Fig. 2. The activation energy calculated from the slope of the line is 3·3 eV. The reported[2] activation energy for the




decomposition of unirradiated lead oxalate is 36·0 *k cal./mole*.

A more detailed account of this work, together with further observations, will be published elsewhere. We wish to thank the South African Council for Scientific and Industrial Research for a grant towards *irradiation costs and for a scholarship* held by one of us (P. J. H.).

P. J. Herley
E. G. Prout

Chemistry Department,
*Rhodes University*,
Grahamstown.
June 29.

[1] Prout, E. G., *Nature*, **183**, 884 (1959).
[2] Bircumshaw, L. L., and Harris, L., *J. Chem. Soc.*, 1898 (1948).
[3] Prout, E. G., *J. Inorg. Nucl. Chem.*, **7**, 368 (1958).
[4] Prout, E. G., and Sole, M. J., *J. Inorg. Nucl. Chem.*, **9**, 232 (1959).

## CHEMISTRY

### Mechanism of Atom Recombination

*The recombination of two atoms requires the* intervention of a third body—a chaperon[1]—which stabilizes the diatomic complex by reducing its energy below that of the two separate atoms. One plausible kinetic representation of this process is

$$I + I \rightleftarrows I_2^* \qquad (1)$$

$$I_2^* + M \rightarrow I_2 + M \qquad (2)$$

where $I$ are atoms, $M$ is the chaperon and $I_2^*$ represents a collision complex which is iso-energetic with the separate atoms.

This scheme does not give a satisfactory account of the principal features of atom recombination reactions *which have been observed experimentally*. The most accurate data are those derived from flash photolysis studies of halogen atom recombinations which have established that: (*a*) rates of recombination of iodine atoms in the five inert gases are in inverse order to those calculated for reaction (2) by means of collision theory[2]; (*b*) rates in many other gases, for example, mesitylene[3] and iodine[4] are much greater than would be predicted simply on the basis of increased collision diameters or additional degrees of freedom; (*c*) temperature coefficients are negative[3].

An alternative mechanism which has been widely discussed for some time (see, for example, refs. 5, 6) is the following:

$$I + M \rightleftarrows IM \qquad (3)$$

$$IM + I \rightarrow I_2 + M \qquad (4)$$

In order to explain the negative temperature coefficients and to account for the efficiencies of the atomic gases, it is necessary to assume that $IM$ is a collision-stabilized complex in thermal equilibrium. *The overall termolecular rate constant of recombination* $k_r$ then becomes equal to $k_4K_3$ where $K_3$ is the equilibrium constant of $IM$ formation. The rate constant can then be expressed in the form:

$$k^0 = AT^{\frac{1}{2}} e^{-\triangle E/RT} \qquad (5)$$

where $\triangle E$ *is the increase in internal energy accompanying* reaction (3) and $A$ is a factor which is nearly independent of temperature. Detailed calculation by statistical mechanical and collisional theory methods shows that the relative rates with the various chaperons *will then be determined principally by the* magnitude of $\triangle E$.

Previous experimental work has not supported this conclusion. The only extensive work on temperature coefficients in a number of gases indicated *that these coefficients were nearly constant for all* chaperons[3]. Only in the case of iodine as the chaperon molecule is there clear evidence for a higher temperature coefficient, but this was thought to be a special case of chemical compound formation[3]. *A further serious difficulty is that energies of formation* of complexes $IM$ calculated from second virial coefficients are too small in absolute magnitude and in relative variation to account for the observed rates.

In order to test the complex theory of recombination *we have recently carried out an investigation* of the temperature coefficients of iodine atom recombination in a number of gases and our results are now sufficient to establish that there is a definite correlation between the rate of recombination and the *magnitude of the negative temperature coefficient*. The values of $A$ and $\triangle E$ defined by equation (5), and the rate constant of recombination at 20° C. are given for iodine atom recombination in nine different gases in the accompanying table, $\triangle E$ for *iodine being taken from ref. 7. The value of A* varies in a random manner by a factor of about ten whilst the rate varies over a factor of 10³. Some variation in $A$ is to be expected and absolute calculations of $A$ require a rather empirical choice of *collisional diameters and statistical weight factors*

Table 1

| Chaperon | $k_{20}$ (ml.² molecules⁻² sec.⁻¹) × 10⁴² | $A$ (ml.² molecules⁻² sec.⁻¹) × 10³⁶ | $-\triangle E$(k.cal./mole) |
|---|---|---|---|
| Helium | 0·84 | 2·6 | 1·4 |
| Argon | 1·64 | 1·8 | 2·0 |
| Oxygen | 3·72 | 2·9 | 2·2 |
| Carbon dioxide | 7·41 | 4·1 | 2·4 |
| Benzene | 43·9 | 24 | 2·4 |
| *Toluene* | *107* | *11* | 3·4 |
| Ethyl iodide | 144 | 24 | 3·1 |
| Mesitylene | 223 | 2·0 | 4·8 |
| Iodine | 764 | 2·9 | 5·3 |

but a not unreasonable selection of such parameters can lead to agreement with the experimental values[9].

If this evidence is regarded as sufficient to establish *the theory outlined we must conclude that the iodine* atom forms complexes with other gases whose energies of formation vary from 1.4 k.cal. for helium to about 5 k.cal. for mesitylene and iodine. These values are considerably greater than the usual estimates of *Van der Waals type interactions between such species*.

Absolute calculations based on such interactions can lead to correct rate constants in some cases[7] but they depend on a fortuitous choice of various

quantities and are of far less significance than the fact that neither the correct magnitude of temperature coefficients nor the correct relative magnitude of the rate constants are predicted. Calculations of $IM$ energies from second virial coefficients are carried through on the assumption that the iodine atom has the same properties as xenon and use of the geometrical mean combining rule. The absolute magnitude of the calculated interaction energy could be increased by assuming that the iodine atom has a much higher polarisability than xenon but no matter what properties of iodine are assumed the observed relative values of $IM$ interaction energies cannot be reproduced.

The phenomena are too general to be interpreted in terms of specific chemical forces and we believe that the explanation is to be found in terms of a charge transfer complex between the iodine atom and the chaperon. No satisfactory quantitative theory of charge transfer forces has yet been developed but the theory of Matsen *et al.*[8], and the limited experimental results on charge transfer complexes involving the iodine molecule in solution indicate that energies between one and five k.cal. are not unreasonable for charge transfer complexes between the gases studied and a species with high electron affinity, such as the iodine atom.

The question as to whether the complex mechanism and the charge transfer theory are generally applicable to other atom recombination reactions and perhaps to some radical recombination reactions must wait further experimental data. Although the energy of formation of charge transfer complexes by other atoms will generally be less than those of iodine, we shall expect that in many other cases the complex mechanism of recombination will play a significant part.

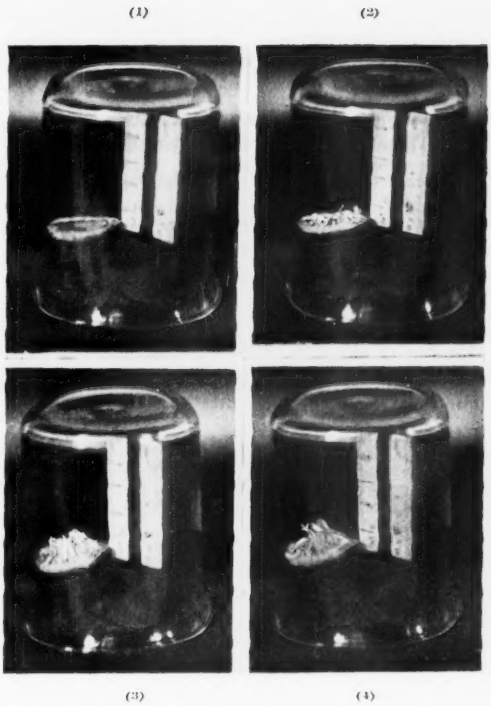
G. Porter
J. A. Smith

Department of Chemistry,
The University,
Sheffield, 10
Aug. 13.

[1] Adam, N. K., "Physical Chemistry", 460 (Oxford Univ. Press, 1956).
[2] Christie, M. I., Norrish, R. G. W. and Porter, G., *Proc. Roy. Soc.* A, **216**, 152 (1953).
[3] Russell, K. E., and Simons, J., *Proc. Roy. Soc.*, A, **217**, 271 (1953)
[4] Christie, M. I., Harrison, A., Norrish, R. G. W., and Porter, G., *Proc. Roy. Soc.*, A, **231**, 446 (1955).
[5] Rice, O. K., *J. Chem. Phys.*, **9**, 258 (1941).
[6] Semenov, N. N., "Some Problems of Chemical Kinetics and Reactivity" (Pergamon Press Ltd., London, 1958).
[7] Bunker, D. L., and Davidson, N., *J. Amer. Chem. Soc.*, **80**, 5085, 5090 (1958).
[8] Hastings, S. H., Franklin, J. L., Schiller, J. C. and Matsen, F. A., *J. Amer. Chem. Soc.*, **75**, 2900 (1953).
[9] Porter, G., and Smith, J. A. (to be published).

## Primer Explosion Triggers Reaction

Recently, when making certain experiments with the fuses for trench mortar shells, a thin (0·1 mm.) disk of pure aluminium was placed between the primer and the detonator. It was noticed that, immediately after this disk was pierced by the gases of the exploding primer, a white mass started to grow on the aluminium surface around the hole, and this growth continued to expand most vigorously for about 12–15 min.




*Figs. 1–4 show consecutive stages of this reaction.* After about 10 min. (Fig. 4) the fragile structure started to collapse under its own weight.

The disk was placed under an inverted beaker to protect the delicate growth from air currents. *In some cases those 'feathers' reached a height of* about 20 mm. before collapsing and resembled greatly *in shape the delicate sea anemones.* The primer charge consisted of, approximately, 50 mgm. of the usual priming mixture containing mercury fulminate, potassium chlorate and antimony sulphide.

The mechanism of this reaction is, apparently, as *follows: vapours of mercury formed at the moment* of explosion ($Hg(CNO)_2 = Hg + 2CO + N_2$), are condensed as tiny droplets on the cold aluminium surface; in presence of the *moisture of air an* energetic electrochemical action starts at those points *where two metals are in intimate contact* and aluminium is readily converted into its hydroxide—$Al(OH)_3$.

G. Larikov

Ordnance Research Institute,
2 Tsinan Road, 1st Section,
Taipei, Taiwan.
June 9.

## A New Approach to Carbon Gasification

Terms such as 'active sites' and 'active centres' have long been used in describing the reactions of carbons with gases, without any specific knowledge of either the real nature or real function of such 'sites'. *Recent work in these Laboratories has thrown some* light on both nature and action of at least one common type of reactive centre.

A series of chars was produced from *Eucalyptus marginata*. These samples contained varying amounts of oxygen depending on the temperature of charring. When this oxygen was determined by heating the char in a stream of purified nitrogen at 1,250° C., it was liberated partly as carbon dioxide and partly as carbon monoxide. The greater part of the oxygen, liberated as carbon monoxide, represents oxygen groupings in

which only one atom of oxygen is attached to a carbon atom. These groups show both oxidizing and reducing characteristics and may possibly be the semiquinone type or the chromene groups described by Garten and Weiss[1,2].

When reacted with hydrogen, carbon dioxide or water vapour, to form methane, carbon monoxide and carbon monoxide plus hydrogen respectively, under the usual conditions of temperature and pressure applying in gasification practice, the rates of reaction measured by the number of gm. moles of product formed per min. per gm. of carbon, were found to follow, in every case, the type of curve shown in Fig. 1.

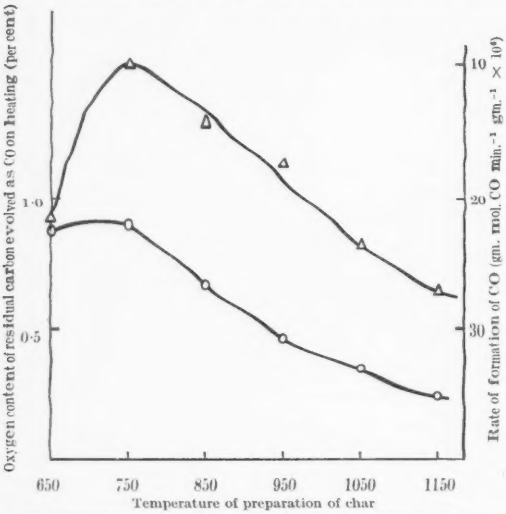



Fig. 1 Showing relationship between residual oxygen bound as ≡C—O— and reaction rate. ○, Oxygen content of char residues; △, reaction rate of carbon when gasified by water vapour dissociated in a radio-frequency field

This pointed to the oxygen-containing groups as being the rate-determining agents.

The same chars were also reacted in an apparatus[3] in which the gases were first passed at a pressure of about 0·3 mm. mercury through a high-frequency field. The effect of the field was to produce high concentrations of hydrogen atoms from hydrogen, oxygen atoms and carbon monoxide from carbon dioxide, and hydrogen atoms and hydronyl radicals from water vapour. In the experiments with hydrogen and carbon dioxide the atomic species reacted very readily with the carbon at room temperature and the rate of each reaction was the same for all the chars, thus indicating that atoms of hydrogen or oxygen were reacting with the carbon independently of the active sites. However, for water vapour the reaction-rates showed the same variation as for the various gases at high temperatures and pressures in the absence of the radio-frequency field.

It is deduced from this evidence that the role of the oxygen groups is to split the molecular species involved to atoms, which then react with the carbon either at the site or nearby. In the cases of hydrogen and carbon dioxide in the high-frequency field the production of atoms of hydrogen and oxygen is brought about by the field and the oxygen groups are therefore not involved. However, for water vapour in the field splitting of the hydroxyl radicals must still be brought about before oxidation can occur, and this is again effected by the oxygen groups.

A more detailed account of this work will be presented elsewhere.

J. D. Blackwood
F. K. McTaggart

Chemical Research Laboratories,
*Commonwealth Scientific and*
Industrial Research Organization,
Fishermen's Bend, Melbourne.

[1] Garten, V. A., and Weiss, D. E., *Aust. J. Chem.*, **8**, 68 (1955).
[2] Garten, V. A., and Weiss, D. E., *Aust. J. Chem.*, **10**, 309 (1957).
[3] Blackwood, J. D., and McTaggart, F. K., *Aust. J. Chem.*, **12**, 114 (1959).

## Polyethylene Absorption Cells for Infra-Red Spectrophotometry

The infra-red spectroscopy of aqueous solutions has been hampered not only by the infra-red absorption of water itself, but also by the difficulty of finding material for absorption cell windows which would be insoluble in water and still sufficiently transparent in the desired region. This problem is particularly irksome to biochemists who are primarily interested in aqueous solutions. Several groups of workers[1-4] have been successful in observing infra-red spectra in water solution by using cells sufficiently thin for absorption by water not to be excessive and by choosing materials such as silver chloride, barium fluoride, thallium bromide, etc., for windows. The expense and inconvenience of these special windows has prevented many from benefiting by this powerful technique, so that we have been led to consider the possibility of using polyethylene as a window material for aqueous solutions.

All measurements have been made using the Perkin–Elmer 'Infracord', Model 137 Spectrophotometer. Two pieces of polyethylene sheet were heat-sealed around three sides to form bags of a size that would fit in the regular sample cell holders. A few drops of liquid to be analysed were added and the excess squeezed out to remove air bubbles and leave a capillary film.

Fig. 1 gives the absorption spectrum of polyethylene showing that large transparent bands are available between the hydrocarbon absorption bands. For comparison a spectrum of 'Nujol' is included since 'Nujol' is frequently used as a mulling medium in spectral determinations. Polyethylene is clearly as transparent as 'Nujol' in the regions of most interest.

Fig 2. compares the absorption spectra of capillary films of nitrobenzene on sodium chloride and polyethylene windows. Except for the narrow bands of polyethylene absorption, the two spectra are identical, so that because of its negligible cost, polyethylene may be preferred even for some compounds which can be run on sodium chloride. No cleaning of polyethylene windows is ever necessary; fresh ones can be used for each determination.

Fig. 3 gives the absorption spectra of sodium acetate as a 'Nujol' mull and in aqueous solution. In the region around 3 microns 'Nujol' appears superior because of the large water absorption. If desired, deuterium oxide rather than water may be used as a solvent[2] so that only the narrow polyethylene band would interfere. In the carboxyl absorption region better resolution was obtained in solution than in 'Nujol' since 'Nujol' has two absorption bands here while polyethylene has one narrow band which falls at 6·8μ, just between the carboxyl bands at 6·4 and 7·1μ. Throughout the remainder of the

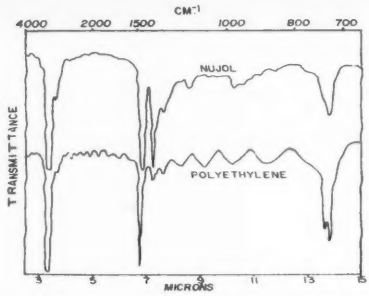



Fig. 1. Infra-red absorption spectra of 'Nujol' in a capillary film on sodium chloride windows and of polyethylene film in two layers each of 0·002 in. thick

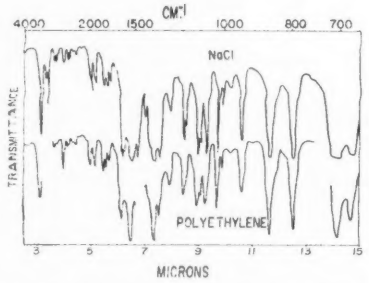



Fig. 2. Infra-red absorption spectrum of nitrobenzene as a capillary film either between sodium chloride windows or between two layers of 0·002 in. polyethylene film. In the latter case a similar thickness of polyethylene was in the reference beam. Breaks in the bottom curve indicate regions which are not usable because of polyethylene absorption

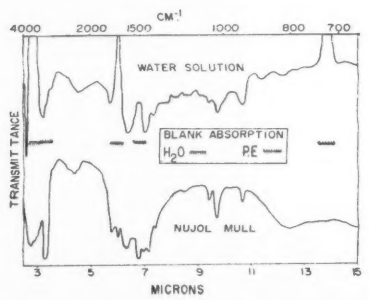



Fig. 3. Infra-red absorption spectrum of sodium acetate trihydrate as a 'Nujol' mull between sodium chloride windows or in saturated aqueous solution as a capillary film between polyethylene sheets. In the latter case the reference beam passed through a capillary film of water in polyethylene. Hatched lines indicate regions where high absorption by water or polyethylene makes sample spectrum indistinguishable

spectrum there is little of interest, but the two techniques appear to give similar bands. Both polyethylene and 'Nujol' show strong absorption at 13·5–14·0μ. The difference appears because for polyethylene this was compensated by a reference sample.

The major objection to the polyethylene technique arises from the difficulty of controlling sample thickness. The flexibility of thin polyethylene prevents spacers being used with any accuracy. However, *for qualitative applications with aqueous solutions* polyethylene seems in many ways preferable to the other cell materials which are available.

Thanks are expressed to the Kordite Corporation, Macedon, New York, for supplying the polyethylene *sheet used in these experiments.*

TREVOR ROBINSON
Biological and Food Research Center,
and
Department of Bacteriology and Botany,
*Syracuse University,*
Syracuse 10, N.Y.
May 11.

[1] Gore, R. C., Barnes, R. B., and Petersen, E., *Anal. Chem.*, **21**, 382 (1949).
[2] *Blout, E. R., and Lenormant, H., J. Opt. Soc. Amer.*, **43**, 1093 (1953).
[3] Potts, W. J., and Wright, N., *Anal. Chem.*, **28**, 1255 (1956).
[4] Ellenbogen, E., Meeting Abstracts, American Chemical Society, September 1959, 24C.

## Stability of Metal Complexes of Oxine and its Sulphonic Acid

THE STABILITY constants for the complexes of 8-hydroxyquinoline-5-sulphonic acid (oxine sulphonic acid) have recently been compared with those for 8-hydroxyquinoline (oxine). For any given metal, the reported difference between corresponding constants was large (up to 10,000-fold) and a plausible explanation was offered[1]. However, the comparison is not valid because the values for the sulphonic acid were determined in water and those of the 8-hydroxyquinoline in 70 per cent dioxan[2].

When a comparison is made between results obtained in the same solvent, it is seen that the presence of the sulphonic acid group has only a small effect on the stability constants. This is clearly shown by the typical values (Table 1), all of which were obtained in water at 20-25°.

TABLE 1

| Cation | Oxine (ref. 3) | Oxine sulphonic acid (ref. 3) | Oxine (ref. 4) | Oxine sulphonic acid (ref. 5) |
|---|---|---|---|---|
| | log $k_1$ | log $k_1$ | log $k_1$ | log $k_1$ |
| $Cu^{2+}$ | 12·5 | 12·5 | 12·6 | 11·5 |
| $Ni^{2+}$ | 9·9 | 10·0 | 9·3 | 9·8 |
| $Co^{2+}$ | 9·1 | 9·2 | 8·7 | 8·8 |
| $Zn^{2+}$ | — | 8·4 | 8·6 | 8·7 |
| $Fe^{2+}$ | 8·0 | 8·4 | — | — |
| $Mn^{2+}$ | 6·8 | 6·6 | — | 6·9 |
| $Mg^{2+}$ | 4·5 | 4·8 | 4·7 | 4·8 |
| Method | Potentiometry | Potentiometry | Spectrometry | Spectrometry |
| Ionic strength | 0·01 | 0·01 | ——→0 | ——→0 |

ADRIEN ALBERT
Department of Medical Chemistry,
Australian National University,
Canberra

REINO NASANEN
Department of Chemistry
University of Helsinki,
Helsinki
Aug. 11.

[1] C. F. Richard, R. L. Gustafson and A. E. Martell, *J. Amer. Chem. Soc.*, **81**, 1033 (1959).
[2] L. E. Maley and D. P. Mellor, *Austral. J. Sci. Res.*, **2**, A, 92 (1949).
[3] A. Albert, *Biochem. J.*, **54**, 646 (1953).
[4] R. Näsänen, *Suomen Kemistilehti*, B, **2**, 11 (1953).
[5] R. Näsänen and E. Uusitalo, *Acta Chem. Scand.*, **8**, 112 (1954).

## BIOCHEMISTRY

### Rivanol, Resin and the *Isolation of Thrombins*

Human, cow, and horse thrombins were quickly fractionated from citrate- or bio-activated prothrombin preparations by precipitation of residual prothrombin and inert protein(s) with the cationic dye 6,9-diamino-2-ethoxyacridine lactate (often referred to as rivanol, available under the trade name 'Ethodin' from the Winthrop Laboratories, New York, New York). This is a general procedure previously applied in purifying γ-globulin[1], β1-metal-combining globulin[2], and cæruloplasmin[3]. A more efficient yet rapid fractionation, however, was achieved by the adsorption of preparations containing thrombin on filter cakes or short columns of '*IRC*-50' ('*XE*-64-Rivanol'), this resin form being prepared by stirring '*XE*-64-$Na^+$' with an excess of rivanol. After the impurities, including other rivanol-soluble proteins, were washed off, the thrombins were eluted with 0·15 *M* calcium chloride. The thrombins were recovered from all eluates by acetone precipitation.

As thrombin sources, prothrombin preparations from the plasmas of various species[4] were activated both autocatalytically[5] and with bioactivators prepared by special treatment of acetone powders of brain tissue from the species to be studied. Five hundred mgm. of the brain powders[6] were first incubated with 10·0 ml. of serum from the same species for 30 min. at 25° C. They were then washed twice with 0·15 *M* magnesium chloride and finally suspended in 10·0 ml. of 0·15 *M* calcium chloride. Within 30 min. one volume of the suspensions completely activated 5 vol. of prothrombin solutions containing about 4,000–5,000 units/ml. After activation the thromboplastin was removed by high-speed centrifugation and the proteins were precipitated with cold acetone.

The batch purification of these thrombin preparations by precipitation of impurities with rivanol was studied in various solutions. Maximum purification was obtained over a broad range of conditions. Rivanol concentrations of 0·1–1·0 per cent, ionic strengths of 0·1 to 0·5, and *p*H's between 7·0 and 9·5 did not affect the degree of purification. Anion type did not influence the fractionation. The only important variable was the protein concentration. Quantitative recoveries of thrombin were obtained when the protein concentrations were held in the range of 1–5 mgm./ml., whereas some losses occurred with more dilute or concentrated solutions. The results in Table 1 demonstrate changes in the specific activities of some citrate- and bio-activated horse, cow, and human prothrombins brought about by the batch precipitation of impurities with rivanol. These fractionations were carried out at 0° C. in 0·05 *M* potassium citrate, *p*H 8·5, which was 0·3 per cent with respect to rivanol. After centrifugation to remove the insoluble impurities, the thrombins were precipitated by adding two volumes of cold (−10° C.) acetone and the precipitates were dissolved in dilute magnesium chloride solutions. The inert proteins were recovered from the dye-protein precipitate by dissolving the material in 0·5 *M* trisodium citrate and then adsorbing the rivanol on '*XE*-64-$Na^+$'.

Table 1. Purification of Thrombins with Rivanol and '*XE*-64-Rivanol'

| Method | Species | Type activation | Specific Activity* | |
|---|---|---|---|---|
| | | | Before purification | After purification |
| Batch Rivanol | Horse | Autocat- | 12,900 | 28,500 |
| | Horse | Bio- | 10,700 | 19,400 |
| | Cow | Autocat- | 17,700 | 28,000 |
| | Human | Bio- | 6,900 | 12,100 |
| XE-64-Rivanol | Horse | Bio- | 8,200 | 30,800 |
| | Cow | Bio- | 5,100 | 61,100 |
| | Human | Bio- | 6,900 | 42,000 |

* 'Iowa' units/mgm. tyrosine (Folin-Ciocalteau).

Subsequent to these preliminary batch experiments, a highly efficient and rapid fractionation technique was devised by superimposing the rivanol precipitation phenomenon on the very effective ion exchange chromatography of thrombin first accomplished by Rasmussen[7]. Up to 12-fold purifications were achieved. '*XE*-64-Rivanol' was washed repeatedly with 0·1 *M* sodium acetate. Filter cakes or columns of the resin 5–7 cm. high were prepared and the thrombin preparations were applied and washed with 0·1 *M* sodium acetate until the effluents were protein-free. The thrombins were then eluted with 0·15 *M* calcium chloride, and the effluent fractions were collected with an automatic device. Only 2–3 hr. were required for the entire procedure, including activation of the prothrombin, separation of the thrombin on '*XE*-64-Rivanol', and, finally, precipitation of the thrombin fraction with acetone. Data indicating the degree of purification of several species of thrombin by this method appear in Table 1. Samples of the horse thrombin containing 8,200 units/mgm. tyrosine, which in quantitative yield was purified to 30,800 units/mgm. tyrosine on the '*XE*-64-Rivanol' column, were also purified independently by the batch rivanol treatment and by ion exchange chromatography[7]. The batch technique gave an increase in specific activity to only 16,800 units/mgm. tyrosine, while chromatography produced a product with only 22,800 units/mgm. tyrosine. To emphasize the great efficiency of the purification method, the activated prothrombin preparations selected for this study were purposely of a very low activity compared to their theoretical maxima[4]. However, except for its rapidity, the author doubts that the '*XE*-64-Rivanol' technique is superior to ion exchange chromatography in the isolation of thrombin prepared from completely activated, homogeneous prothrombin preparations[8]. Preliminary studies indicate that the horse and cow thrombins isolated using short '*XE*-64-Rivanol' columns approach homogeneity. If this should be true, it is noteworthy that a twofold difference in specific activities exists between the isolated thrombins of the two species just as between the respective prothrombins[4].

The '*XE*-64-Rivanol' fractionation method is being applied to other protein mixtures, and the several factors governing the separation process are under study.

This work was supported in part by the Office of the Surgeon General, Department of the Army.

Kent D. Miller

Division of Laboratories and Research,
New York State Department of Health,
Albany, New York.

[1] Horejsi, J., and Smetana, R., *Acta. Med. Scand.*, **155**, 65 (1956).
[2] Boettcher, E. W., Kistler, P., and Nitschmann, Hs., *Nature*, **181**, 490 (1958).
[3] Steinbuch, M., and Quentin, M., *Nature*, **183**, 323 (1959).
[4] Miller, K. D., and McGarrahan, J., Proceedings, Spring Meeting, Amer. Chem. Soc., Boston, Mass. (1959).
[5] Seegers, W. H., *Proc. Soc. Exp. Biol. Med.*, **72**, 677 (1949).
[6] Quick, A. J., *Science*, **92**, 113 (1940).
[7] Rasmussen, P., *Biochim. Biophys. Acta*, **16**, 157 (1955).
[8] Miller, K. D., *Fed. Proc.*, **17**, 276 (1958).

## Identification of Phenazocine, a Potent New Analgesic

THE synthesis of a new analgesic of remarkable potency, phenazocine (2-hydroxy-5,9-dimethyl-2-phenethyl-6,7-benzomorphan), has recently been announced[1]. This compound, of which the (−) isomer has an analgesic effect twenty times as great as that of morphine, is now undergoing clinical trials in the United States.

The absolute identification of a compound of this type is of considerable importance, as all synthetic analgesics previously described have been shown to be habit forming, and have therefore been placed under international control. Phenazocine may be identified by both colour and crystal tests[2]. It gives a brown colour with the formaldehyde/sulphuric acid reagent (Marquis) and a bright blue turning to yellow green with the ammonium molybdate/sulphuric acid reagent. It resembles the morphine alkaloids in giving a yellow colour followed by orange with Vitali's test, while with the micro-diazo test[3], when coupled with diazotized *p*-nitroaniline, it gives a brown colour, turning to bluish grey as the test drop dries. These tests, however, do not serve to differentiate between the racemic and the optically active forms of phenazocine, nor to distinguish this compound from 2-hydroxy-2,5,9-trimethyl-6,7-benzomorphan, which also has analgesic properties[4]. Nevertheless, this may readily be done by means of crystal tests. With potassium iodide solution (±) phenazocine gives oily rosettes, the (−) isomer an oily amorphous precipitate, and the trimethyl compound no precipitate at all. With sodium carbonate solution they give bunches of irregular prisms, fans of oily needles, and dense rosettes of prisms respectively. The first two crystallize slowly and incompletely, while crystals of the last form in a few minutes. With picrolonic acid (±) phenazocine gives an oily precipitate, (−) phenazocine shell-like rosettes, and the trimethyl compound curving blades that are highly characteristic.

Run as a paper chromatogram, using the butanol-citric acid system described by Curry and Powell[5], phenazocine has an $R_F$ value of 0·80 and 2-hydroxy-2,5,9-trimethyl-6,7-benzomorphan a value of 0·45

I am grateful to Dr. N. B. Eddy and Dr. E. L. May of the National Institutes of Health, Bethesda, for a gift of the above compounds.

E. G. C. CLARKE

Department of Physiology,
Royal Veterinary College,
London, N.W.1.

[1] May, E. L., and Eddy, N. B., *J. Org. Chem.*, **24**, 294 (1959).
[2] Clarke, E. G. C., and Williams, M., *J. Pharm. Pharmacol.*, **7**, 255 (1955)
[3] Clarke, E. G. C., *J. Pharm. Pharmacol.*, **10**, 194 (1958).
[4] May, E. L., and Fry, E. M., *J. Org. Chem.*, **22**, 1366 (1957).
[5] Curry, A. S., and Powell, H., *Nature*, **173**, 1143 (1954).

## Corticosterone Inhibition of Pyridine Nucleotide Oxidase from Heart Sarcosomes

IT has recently been found in this laboratory that corticosterone is present in higher concentrations in heart tissue than in plasma[1]. Since steroid hormones are known to interfere with tissue oxidations, presumably at the level of the flavine enzymes[2], the relative abundance of corticosterone in heart tissue suggested a study of the effect of this steroid on heart tissue oxidations *in vitro*. Reduced diphosphopyridine nucleotide was chosen as substrate in order to get the flavine enzymes involved as directly as possible. The enzyme preparation used in the experiments was made from isolated pig heart sarcosomes. These were ground with alumina oxide, suspended in dilute tris-buffer *p*H 7·4 and centrifuged for 20 min. at 25,000 *g*. The opalescent supernatant contained an active reduced diphosphopyridine nucleotide-oxidase[3] with a specific activity of about 0·1 μmole reduced diphosphopyridine nucleotide oxidized per min. per mgm. protein at 25° C.

The time course of the oxidation of reduced diphosphopyridine nucleotide is presented in Fig. 1. Addition of corticosterone to a final concentration of $10^{-4}$ *M* produces an instantaneous fall in the rate of oxidation as measured by the decrease in optical density at 340 mμ of the reaction mixture. The reaction product was diphosphopyridine nucleotide also when corticosterone was present. This could be shown by the restoration of optical density to the initial value following addition of the diphosphopyridine nucleotide-specific alcohol dehydrogenase and ethanol.

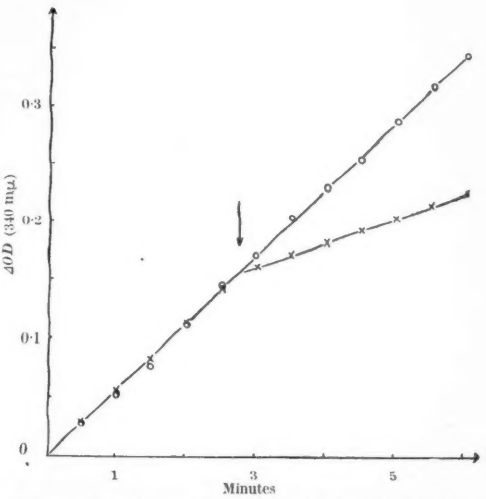



Fig. 1. Corticosterone inhibiton of reduced diphosphopyridine nucleotide-oxidase. Each cuvette contained 25 μmole *tris*/hydrochloric acid, 0·25 μmole reduced diphosphopyridine nucleotide and enzyme in a total volume of 2·5 ml. *p*H 7·4, 25° C. The reaction was started by addition of the enzyme. The arrow indicates addition of 0·25 μmole corticosterone dissolved in 10 μl. dioxane to the experimental, and of 10 μl. dioxane to the control cuvette. Each point represents the mean of changes in optical density recorded in two separate runs. O——O, control; ×——×, corticosterone

Cytochrome *c* is commonly agreed to be a component of the reduced diphosphopyridine nucleotide-oxidase system[4], linked with the dehydrogenation of reduced diphosphopyridine nucleotide by the cytochrome *c* reductase. The effect of corticosterone on the reduction of cytochrome *c* was therefore compared with the effect on the complete oxidase system. A comparison was also made with the effect of corticosterone on the diaphorase activity of the enzyme preparation. The results are given in Table 1. They show that cytochrome *c* reductase is inhibited to about the same extent as the reduced diphosphopyridine nucleotide-oxidase within the range of corticosterone concentrations used. The diaphorase activity, however, is almost unimpaired by the addition of the steroid. These results thus agree with the above-mentioned suggestion that the site of action of the steroid hormones in the respiratory chain lies between the flavoproteins and cytochrome *c*.

Table 1. EFFECT OF CORTICOSTERONE ON REDUCED DIPHOSPHOPYRIDINE NUCLEOTIDE-OXIDASE, CYTOCHROME *c* REDUCTASE AND DIAPHORASE

| Corticosterone conc. (*M*) | Activity = $\Delta OD$ per min. DPNH-oxidase* (340 mμ) | Cytochrome *c* reductase† (550 mμ) | Diaphorase‡ (600 mμ) |
|---|---|---|---|
| | (Figures in brackets are % inhibition) | | |
| 0 | 0·125 — | 0·111 — | 0·182 — |
| $5 \times 10^{-6}$ | 0·108 (14) | 0·103 ( 7) | 0·179 ( 2) |
| $5 \times 10^{-5}$ | 0·067 (46) | 0·057 (49) | 0·164 (10) |
| $5 \times 10^{-4}$ | 0·017 (87) | 0·033 (70) | 0·163 (10) |

* Experimental conditions as stated in legend to figure, except that corticosterone/dioxane was present from the start of the reaction.
† Same as for reduced diphosphopyridine nucleotide-oxidase with the addition of 2·5 μmole potassium cyanide and 1 mgm. cytochrome *c*.
‡ Same as for reduced diphosphopyridine nucleotide-oxidase with the addition of 2·5 μmole potassium cyanide and 0·1 μmole 2,6-dichlorophenol indophenol.

Table 2. EFFECT OF CORTICOSTEROIDS ON REDUCED DIPHOSPHOPYRIDINE NUCLEOTIDE-OXIDASE

| Corticosteroid added (Final conc. $10^{-4}$ *M*.) | Activity = $\Delta OD$ per min. (Figures in brackets are % inhibition) |
|---|---|
| — | 0·110 — |
| Corticosterone | 0·045 (59) |
| 17-hydroxy, corticosterone (cortisol) | 0·070 (36) |
| 11-desoxycorticosterone | 0·045 (59) |
| 17-hydroxy, 11-desoxycorticosterone | 0·070 (36) |
| 17-hydroxy, 11-dehydrocorticosterone (cortisone) | 0·088 (20) |

Experimental conditions as stated in legends to Fig. 1 and Table 1.

Table 2 lists the effect of five different corticosteroids on the oxidation of reduced diphosphopyridine nucleotide. Corticosterone and desoxycorticosterone inhibit the reaction to the same extent. The corresponding 17-hydroxy compounds are less potent inhibitors, and cortisone is the least effective. Mahler *et al.*[5] found that a purified and soluble cytochrome *c* reductase from pig heart was inhibited to 70 per cent after 25-min. preincubation with desoxycorticosterone ($3 \times 10^{-3}$ *M*) but found no effect of cortisone under the same conditions. Both steroids were ineffective in concentrations of $3 \times 10^{-4}$ *M*. The difference between this purified preparation and the preparation used in the present experiments with respect to steroid sensitivity may be explained by the particulate nature of the latter, an explanation offered[5] to account for the difference in sensitivity to British antilewisite and antimycin between the purified preparation of Mahler and a particulate preparation of Slater[4] similar to the one described here.

This work was supported by grants from Statens Alm. Videnskabsfond and Johann og Hanne Wiliemanns Legat.

P. K. JENSEN

Division of Endocrinology,
University Institute for Experimental Medicine
Copenhagen. June 8.

[1] Jensen, P. K., and Bojesen, E., *Abstr. Fourth Int. Congress of Biochem.* Sect. 9-115, Wien (Sept. 1958).
[2] Hayano, M., Dorfman, R. I., and Yamada, E. Y., *J. Biol. Chem.*, **186**, 603 (1950).
[3] Baltscheffsky, H., *Exp. Cell Res.*, **13**, 630 (1958).
[4] Slater, E. C., *Biochem. J.*, **46**, 484 (1950).
[5] Vernon, L. P., Mahler, R. H., and Sarkar, N. K., *J. Biol. Chem.*, **199**, 599 (1952).

## Interferon produced by Cultures of Calf Kidney Cells

CERTAIN influenza *A* viruses multiply readily in bovine kidney cells[1]. Calf kidney cells infected with the influenza *A* strain *WS* produce virus continuously for about 2 days and then cease to do so. Few cells degenerate. After a few more days influenza virus begins to appear again (Fig. 1). Periods in which the amount of virus formed rises and falls may alternate in this way for up to three months. Cultures, infected 6–12 days previously, which were yielding no detectable influenza virus hæmagglutinins, were found to be resistant to superinfection with Sendai virus. No hæmagglutinin was produced and no degeneration occurred.

The development of this state of interference was studied further. It was found that medium collected between 24 and 48 hr. after infection with about 1 hæmagglutinating (*HA*) unit of live influenza *A* contained a substance resembling the interferon of Isaacs and Lindenmann[2]. An experiment demonstrating this is shown in the first column of Table 1. Roller-tube cultures of calf kidney cells maintained in Hanks' saline were infected with approximately 2 hæmagglutinating units of influenza *A* (1947) strain Kunz. Two days later the medium was collected and dialysed for 4 hr. against 0·1*M* citric acid-citrate buffer *p*H 2·1 and then against three changes of buffered saline[3]. This treated medium did not hæmagglutinate and was non-infectious. It was inoculated in volumes of 1 ml. to each of a further set of calf kidney cultures. After 24 hr. the medium in these was completely changed and 0·3 hæmagglutinating units of Sendai virus was inoculated. The medium was titrated 3 days later and the results are given in Table 1. They show a reduction in the amount of virus produced by cells treated with the interferon preparation. The activity of interferon was not eliminated by immune serum against the virus strain used but was destroyed by boiling.

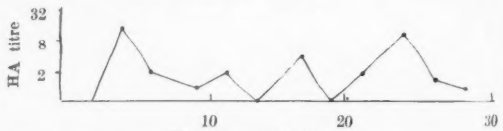



Fig. 1. Titre of hæmagglutinin in a culture tube of calf kidney cells infected with 0·02 hæmagglutinating unit of *WS* virus. The medium contained 5 per cent horse serum and 0·5 per cent lactalbumin hydrolysate and was changed and titrated on the days indicated by the points on the graph.

It was found that interferon could be demonstrated after infection of pieces of chorioallantoic membrane with living Kunz virus using the same technique as in the experiments with calf kidney (column 2 of Table 1). Isaacs and Lindenmann[1] used inactivated virus when they first demonstrated interferon but Burke and Isaacs[3] have since used live virus as described here. However, Table 1 shows that interferon made in cultures of chorioallantoic membrane has much less activity when assayed in calf kidney cells than when assayed in chorioallantoic membrane. Similarly, interferon made in calf kidney cells is relatively more active in homologous than in heterologous cells. It has previously been shown that interferon made with inactivated virus in chorioallantoic membrane has a little activity when assayed in monkey kidney cell cultures.

In view of these results any theory of the nature of interferon should now include an explanation of why it carries some of the specificity of the cell type from

Table 1. INTERFERON ASSAYS ON FLUIDS FROM CULTURES INFECTED WITH INFLUENZA *A* VIRUS.

| Culture used as source of interferon | $Log_2$ mean hæmagglutinin titre in assays using culture of: Calf kidney cells | Chorioallantoic membrane |
|---|---|---|
| Calf kidney cells | 0·3 | 4·7 |
| Chorioallantoic membrane | 5·6 | −1 |
| Saline control | 4·6 | 5·0 |

which it was formed although it is produced as a response to virus infection. Also, interferon production should be considered a possible mechanism by which active virus and cell populations may coexist over long periods of time.

D. A. J. Tyrrell

Medical Research Council Common Cold Research Unit,
Harvard Hospital, Salisbury, Wilts.

1 Heath, R. B., and Tyrrell, D. A. J., *Arch.ges.Virusforsch.*, **8**, 577 (1958).
2 Isaacs, A., and Lindenmann, J., *Proc. Roy. Soc., B.*, **147**, 258 (1957).
3 Burke, D. C., and Isaacs, A., *Brit. J. Exp. Path.*, **39**, 452 (1958).

## PHYSIOLOGY

### Resistance to Flow in Vascular Beds

Carlill's attempt[1] to dispel confusion in the use of the Ohm's law analogy in blood circulation theory requires further clarification, since it is only the incorrect application of this analogy that needs to be abandoned not the analogy itself, but it raises an important point. This is confirmed by Burton[2] when he insists that, for vascular circuits:

$$\text{Resistance to flow } (R) = \frac{\text{Pressure drop across the bed } (P)}{\text{Flow } (F)}$$

For a definition consistent with the electrical analogy we must have $R = (P-p)/F$ where $p$ is the critical closing pressure as referred to by Carlill. This gives $F = P/R - p/R$, which is of the form $y = mx - c$ since $p/R$ is constant for any single one of the lines in Carlill's Fig. 1 and gives the same result for $dF/dP$ as in Burton's equation (4) derived from his incomplete expression of form $y = mx$.

The usage of the term 'dynamic resistance' is well defined in electrical fields, as is the term 'ohmic resistance', and it is desirable that analogous applications of such terms in physiological fields should be equivalent to the electrical ones. If conventional practice in electrical analysis (that is, analytical symbols represent pure properties, for example, of voltage, resistance, rectification, as distinct from practical components which usually combine several properties) be used to interpret Carlill's Fig. 1, it can be seen that the lines showing pressure intercepts have characteristics given, in electrical analogy, by a direct current source, or battery, in series with a resistance obeying Ohm's law and a rectifier. This equivalent circuit is shown in Fig. 1 between points $A$ and $D$.

For current flow to occur (in fashion comparable with the vascular case) an electrical (hydraulic) energy source (of magnitude $P$), as shown between $X$ and $Y$, must be acting across $AD$ (or the vascular bed). The voltage between $A$ and $B$ (equivalent to the critical closing pressure $p$ of the vascular bed, and of magnitude specified by Carlill's pressure intercept) opposes the flow of electricity (or of blood or perfusion fluid). The term 'dynamic resistance' then refers to measurements made across $AC$ or $AD$ using a number of applied voltages (or pressures), which could involve alternating current (pulsating flow), and is obtained as a single value, the reciprocal slope of a straight line, so long as resistance $BC$ is ohmic. If resistance $BC$ is non-ohmic, say having a partly curved characteristic as instanced by Burton, then a number of values of dynamic resistance must be chosen for parts of the curve, each of which is regarded as approximating to a straight line. Carlill only considers the case where resistance $BC$ does follow Ohm's law. Clearly the Ohm's law analogy is applicable to specify the resistance component of the vascular beds considered by him and is very helpful, if not essential, in explaining and defining the term 'dynamic resistance'. A more extensive discussion of this type of electrical analysis and of the term 'dynamic resistance' is given by Richter[3]. It shows how the confusion in physiological theory to which Carlill draws attention can arise in electrical theory in precisely analogous fashion.

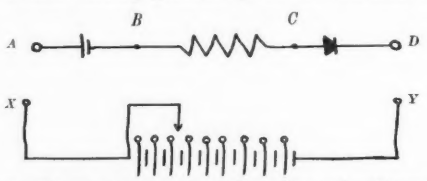



Fig. 1. This shows, between $A$ and $C$, a voltage and resistance as electrical analogies of the two components proposed by Carlill[1] to specify 'resistance to flow' in the vascular beds related to his Fig. 1, namely 'pressure intercept' and 'dynamic resistance'. The rectifier between $C$ and $D$ is necessary to complete the analysis of Carlill's lines showing pressure intercepts. Between $X$ and $Y$ is a variable voltage source, the electrical analogy of the hydraulic energy source which must be applied across a vascular bed to determine its critical closing pressure and dynamic resistance

Carlill proposes only the use of pressure and resistance components as represented between $A$ and $C$. However, connecting $A$ to $C$ would give a current flow. Also a reduction of pressure to negative values, that is, application of a suction, cannot produce a reversal of flow in the vascular bed. This 'valve effect' in a vascular bed must be represented electrically as the rectifier shown between $C$ and $D$. The analogy with the effect of a single rectifier is virtually complete for vascular beds containing veins with competent valves, which permit blood flow in only one direction under positive pressures in addition to the valve effect of the closed vessels under negative pressures or small positive ones.

For vascular beds without vein valves, or with incompetent ones, an analagous electrical circuit may be obtained by adding in parallel with the circuit between $A$ and $D$ in Fig. 1, its own mirror image making contact $D'$ with $A$ and $A'$ with $D$, or by drawing a full-wave rectifier bridge with a battery and resistance in series across its output.

In the above equivalent circuits an ohmic resistance component can be said to be involved, although the characteristic of the complete circuit over the range giving analogy with vascular phenomena, as far as it goes, departs from Ohm's law.

In practice the circuit between $A$ and $D$ in Fig. 1 can be replaced by an appropriately designed triode valve having mutual characteristics corresponding with the pressure-flow lines of Carlill's Fig. 1. The magnitude of the pressure intercept for any of the pressure-flow lines is then determined in the triode analogy by the grid bias voltage. Other practical analogues can also be found.

The above analysis does not cover all details of the non-ohmic properties found in vascular circuits, some of which were dealt with by Burton, but is confined to those arising from the phenomena which Carlill is concerned to analyse. It has been necessary to assume simplified vascular circuits, corresponding with the electrical circuits mentioned, in order to achieve the necessary link with his treatment. A comprehensive treatment could usefully lead to the design of practical analogue circuits giving models of the circulation, or of parts of it, impossible to achieve by mechanical means.

The crux of this problem seems to lie in the different levels of advancement of analytical theory in the fields of electronics and physiology of the circulation. The development of physiological concepts now taking place should follow analogous practice in electrical or other fields where prior development has occurred, if circulatory physiology is to benefit from the possible uses of electrical analogues.

Harris S. Burry

The British Boot, Shoe and Allied Trades,
Research Association,
Satra House,
Rockingham Road,
Kettering,
Northants.

1 Carlill, S. D., *Nature*, **181**, 1607 (1958).
2 Burton, A. C., *Nature*, **182**, 1456 (1958).
3 Richter, W., "Fundamentals of Industrial Electronic Circuits", Chap. 5. (McGraw-Hill, 1947).

## Interrelations between the Blood Coagulating System and the Physiological Anticoagulating System

Previous publications[1,2] attest to the existence of a physiological anticoagulating system in some animals. Moreover, a surmise was made concerning the presence *in blood vessels of chemoreceptors reacting to the* appearance of thrombin in the blood, and through the reflex are calling into play a neuro-humoral mechanism which prevents the coagulation of the circulating blood.

In subsequent experiments carried out by Kalishevsky and me, the physiological anticoagulating system was found to exist not only in the organism of mammals, but also in that of amphibians.

After injecting into the ventricular cavity of the frog's heart a moderate dose of thrombin (0·35–0·40 ml. clotting an equal volume of the frog's blood at 37° in 7–9 sec.), the circulation of blood was maintained and no clots were formed. Blood taken from the hearts of such animals completely lost its coagulating capacity *in vitro* in the presence of thromboplastin obtained from the tissue of the frog's lungs. At the same time, in frogs with a preliminarily destroyed spinal cord the injection of the same dose of thrombin in all cases resulted in an immediate total coagulation of blood in the vascular system. The destruction on extirpation of the brain as distinct from destruction of the spinal cord did not have the same effect, the anticoagulating system of such animals remaining in its functional condition. This suggests that the centre of the frog's reflex are receiving the signal of the appearance of thrombin in the circulating blood is connected with the spinal cord.

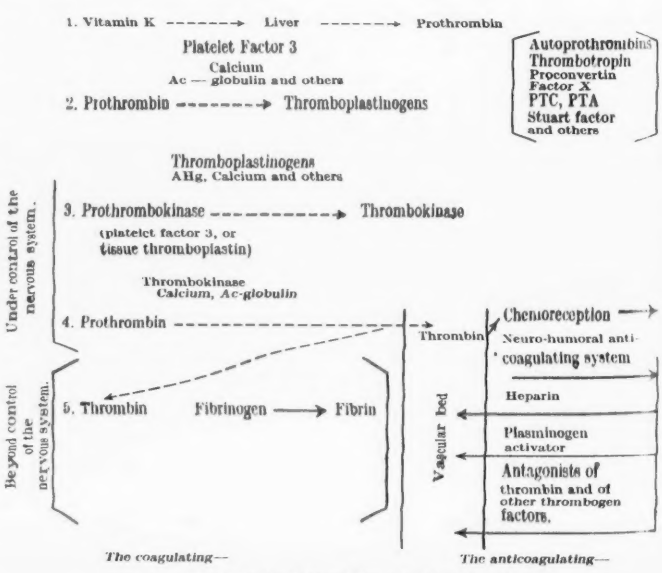
*In previous communications*[1,2] *it* was shown that in response to a signal of thrombin formation in the circulating blood transmitted by chemoreceptors present in the vascular system, certain agents are immediately given off into the blood to prevent its coagulation, namely, fibrinolytic and heparin-like substances.

In experiments carried out on frogs, it was established that a preliminary injection of heparin (0·1 ml. in concentration of 1/100,000) into the blood of frogs with destroyed spinal cords fully protected these animals against an intravascular blood clotting after a subsequent injection of thrombin solution into their heart cavities.

Under the same conditions the blood of normal frogs was found to possess the same protective property after thrombin injection into it. Thus, 0·9 ml. of such a blood saved a frog the spinal cord of which had been destroyed from thromb-formation, when followed by an injection into its heart of 0·35 ml. of thrombin solution clotting *in vitro* the normal frog's blood in 9 sec. at 37°.

Experiments made in collaboration with Pastorova have shown the blood of rats to become greatly enriched with active plasmin after an intravenous *infusion of tissue thromboplastin or thrombin, due* to which a sharply accelerated fibrinolysis is seen to occur in distinction from the normal control animal blood. These results serve to prove that in a reflex act induced by the appearance of thrombin in the *circulating blood, an activator of plasminogen* together with other substances, is produced in the blood. This fact causes the formation of an excess of plasmin which removes fibrinogen.

The various schemes already put forward with the aim of interpreting the biochemical mechanism of blood coagulation without taking into account the existing *neuro-humoral regulation, are, to a greater of lesser* degree, correct for the process occuring *in vitro* or outside a normal vascular bed. The foregoing results show that the appearance of thrombin in the blood may lead to diametrically opposite reactions *depending upon the presence or absence of the nervous* system control. If thrombin appears in the circulating blood under physiological neuro-humoral control, it does not produce coagulation, but on the contrary




parts of the blood coagulation physiological mechanism.

its presence results in 'switching off' the clotting mechanism. When thrombin is formed in the blood which is beyond the control of the mechanism indicated, a conversion of fibrinogen to fibrin is seen to occur. That process may occur on the surface of the wound or in a test-tube as well as in the vascular system in pathological cases inducing a dysfunction of the physiological anticoagulating system.

The coagulation and the anticoagulating physiological mechanisms should be considered as two opposite but inseparably connected parts of a single clotting system of blood. This may be illustrated by the accompanying scheme.

The scheme indicates only some essential steps in the clotting process and of its regulation. To avoid unnecessary complication the actually existing two-stage slow and rapid form of thrombin production[3] is omitted. At the appearance of thrombin the regulatory mechanism of the neuro-humoral anticoagulating system already seems to act during the former slow stage, thus preventing an excessive thrombin content being present in the circulating blood. The scheme is based partly on Seegers' results[4,5] on transformation of prothrombin into autoprothrombins, that is, into agents stimulating the conversion of prothrombin to thrombin. The scheme indicates natural coagulants stabilizing the liquid condition of the circulating blood as a dynamic system, in contradistinction to suggestions that there is a static balance between coagulating and anticoagulating components.

B. A. Kudrjashov

Faculty of Biology and Soil Science,
Moscow State University.

[1] Kudrjashov, B. A., and Ulytina, P. D., *Dokl. Akad. Nauk U.S.S.R.*, **120**, 677 (1958).
[2] Kudrjashov, B. A., and Ulytina, P. D., *Nature*, **182**, 397 (1958).
[3] Stefanini, M., and Dameshek, W., "The Hemorrhagic Disorders" (London, 1955).
[4] Seegers, W. H., and Johnson, S. A., *Amer. J. Physiol.*, **184**, 259 (1956).
[5] Seegers, W. H., and Landaburu, R. H., *Amer. J. Physiol.*, **191**, 167 (1957).

## Response of a Single Retinula Cell to Polarized Light

It appears to be a well established fact that some arthropods use polarized light as a kind of light compass. von Frisch[1] has shown clearly that honey bee workers utilize polarized light from the blue sky to identify the direction of a food source. Wellington[2] reported that adult flies can also exhibit orientation relative to the direction of vibration of polarized light. These reports suggest that the arthropod photoreceptor, both the compound eye and the ocelli, has a kind of polarizer in the visual organ. Wulff[3] has reviewed the many attempts that have been made to localize the possible polarizer in the compound eye, and to find the physiological mechanism involved in the reception of polarized light in the compound eye, but no conclusion on this problem seems to have been reached.

Kuwabara and Naka[4] have recorded an intracellular action potential from the compound eye of the fly and conclude that the response was obtained from a retinula cell. In our experiments the effects of stimulation by polarized light on the intracellularly recorded action potential were observed.

The fly, *Lucilia caeser*, reared in this laboratory, was used. As shown in Fig. 1 the stimulating apparatus consisted of a 250-watt projector lamp, a

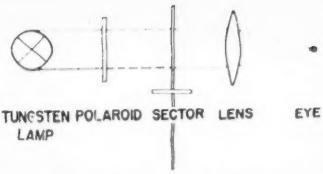


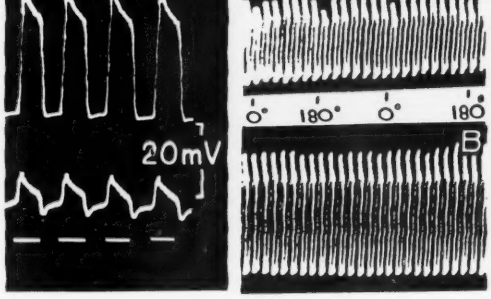

Fig. 1. Schematic representation of the optical apparatus to give intermittent polarized light. The polaroid was rotated at 15 r.p.m. while the sector interrupted the polarized light to give intermittent stimulation at 3 cycles per second.

Mitsubish 'Diachrom' polaroid, and a turning sector to give an intermittent stimulation of about 3 cycles per second. In the experiment the polaroid was continuously rotated at about 15 r.p.m. while the turning sector interrupted the polarized light to take a continuous recording of responses. Other experimental procedures were the same as described elsewhere.[5]

In Fig. 2 intra- and extra-cellular responses to the stimulation at 3 cycles per second are shown. The former were monophasic waves with an amplitude of more than 40 mV., while the latter were diphasic with amplitude of about 5 mV. This figure also shows that the intracellularly recorded response completely followed stimulation at 3 cycles per second. The response to polarized light is shown in Fig. 3*A* and the record in Fig. 3*B* is a control taken without the polaroid. From these records it is clear that the amplitudes of the action potentials varied synchronously with rotation of the polaroid, whereas in the control these amplitudes remained the same throughout the stimulation. The amplitude of the action potential decreased about 20 per cent when the plane of polarized light was rotated through 90°.

As these records were obtained intracellularly from the receptor layer, the response to polarized light in Fig. 3 appears to represent the response of a single receptor cell, that is the retinula cell.

The recent electron-microscopic studies on the microstructure of the insect compound eye have revealed that the rhabdomere is composed of many regularly arranged honeycomb like microvili, and that the direction of the arrangement of the honeycomb-like structure is different in each rhabdomere[6]. In an ommatidium of the fly there are seven retinula cells




(2) (3)

Fig. 2. Intracellular (upper record) and extracellular (lower record) responses to intermittent stimulation at 3 cycles per second.
Fig. 3. *A*, intracellularly recorded responses to the stimulation by polarized light; *B*, control recorded without polaroid. Amplification is the same as in Fig. 2.

and each retinula cell has a rhabdomere, indicating that any stimulation of a retinula cell is mediated by the rhabdomere attached to the cell. Thus the electron-microscopic studies and the results of the present experiment favour the view that the rhabdomere acts as a polarizer in the compound eye.

M. Kuwabara
K. Naka

Department of Biology,
*Kyushu University*,
Fukuoka, Japan.
May 10.

[1] von Frisch, K., "Bees, Their Vision, Chemical Senses and Language", (Cornell Univ. Press, Ithaca, 1950).
[2] Wellington, W. G., *Nature*, **172**, 1177 (1953).
[3] Wulff, V. J., *Physiol. Rev.*, **36**, 145 (1956).
[4] Kuwabara, M., and Naka, K., *J. exp. biol.*, (in the press).
[5] Naka, K., and Kuwabara, M., *J. ins. Physiol.*, **3**, 41 (1959).
[6] Fernandes-Moran, H., *Nature*, **177**, 742 (1956). Wolken, J. J., Capenos, J., and Turano, A., *J. Biophys. Biochem. Cytol.*, **3**, 441 (1957). Goldsmith, T. H. and Philpott, D. E., *ibid.*, **3**, 429 (1957).

## A Low Concentration of Certain Blood Constituents observed in Offspring of Alloxan-Diabetic Rabbits

The purpose of this communication is to report our observation of low levels of plasma cholesterol, albumin and protein-bound hexose in offspring of alloxan-diabetic rabbits on the first day of life.

Four female rabbits of no particular strain weighing approximately 3,500 gm. were rendered diabetic by injecting alloxan monohydrate intravenously in an amount of 200 mgm./kgm. body-weight. The animals were given protamine zinc insulin and, when the metabolic condition was stabilized and no signs of albuminuria were present, they were mated with normal males. The rabbits were fed commercial chow *ad libitum* and supplements of carrots and lettuce were provided. Once a month a multiple vitamin preparation was administered intramuscularly. On this regimen the blood sugar-levels fluctuated about the 250 mgm. per cent level and the glycosuria seldom exceeded 10 gm. a day. This was the best control which we were able to achieve without encountering the danger of hypoglycæmic attacks. Under these conditions demands for exogenous insulin fluctuated widely in pregnant as well as non-pregnant animals. Four normal rabbits were bred and maintained under the same experimental conditions.

The length of pregnancy and the weights of new-borns are recorded in Table 1. Two or more animals from each litter were killed within the first 18 hr. of life by severing the carotid arteries, and blood was collected using heparin. Results of chemical analyses are presented in Table 2. The remaining animals of each litter were killed in the same fashion on the second and third day of life, but results of chemical analyses are not presented. Offspring of diabetic as well as of normal females appeared equally lively and well developed on the first day of life, and the stomachs of all autopsied new-borns were filled with milk. It is therefore unlikely that the differences observed between new-borns of diabetic and normal mothers would be the result of inadequate dietary intake in the postnatal period. On the second and third day of life, many new-borns of the diabetic animals deteriorated rapidly. Their stomachs were found empty, and lack of maternal care was evident in many other ways. This invalidated further comparisons between offspring of normal and diabetic mothers after the first day of life.

Blood sugar was determined on whole blood by King's method[1]. Plasma cholesterol was estimated by the Bloor, Pelkan and Allen procedure[2] and protein-bound hexose by Lustig and Langer's method[3] with minor modifications. Total plasma protein was determined with the biuret reagent by Weichselbaum's method[4]. Fractionation of plasma proteins was carried out by paper electrophoresis by Kunkel's method[5]. Paper strips were stained with bromophenol blue and cut into appropriate segments. The dye was eluted and the intensity measured in a photoelectric colorimeter. The carbohydrate components were visualized by staining paper strips with a modified periodic acid Schiff reagent, following Köiw and Grönwall[6].

It is noteworthy that a high incidence of still-birth and intra-uterine death observed by Miller[7] has not been encountered under our controlled experimental conditions. The length of pregnancy in our diabetic animals was not different from that observed in the normals, but the offspring of the former weighed less. At the same time there was statistically a highly significant difference between corresponding levels of total cholesterol, protein-bound hexose and albumin in the two groups of new-borns. The lowering of the total levels of plasma protein was probably a reflexion of low albumin-levels in offspring of diabetic rabbits because no appreciable differences were found in the three globulin fractions. Similarly, no appreciable differences were found in blood-sugar levels between the two groups of new-borns. No corrections were made on the basis of hæmatocrit readings, but it was obvious that the pronounced tendency toward hæmoconcentration in the offspring of diabetic females made the observed differences even more significant.

Table 1. Results of Pregnancies in Normal and Diabetic Rabbits

| Observation | Normal | Diabetic | *P* value | Significance |
|---|---|---|---|---|
| No. of animals | 4 | 4 | — | — |
| No. of pregnancies | 4 | 4 | — | — |
| Length of pregnancy (mean ± S.D.) | 32·0 ± 0·8 | 31·8 ± 1·0 | > 0·5 | No |
| Total No. of live new-borns | 35 | 31 | — | — |
| Dead | 2 | 1 | — | — |
| Weights of live new-borns (mean ± S.D.) | 53·8 ± 13·0 | 43·6 ± 6·1 | < 0·01 | Yes |

Table 2. Offspring of Normal and Diabetic Rabbits: Blood Chemistry on First Day of Life

| Constituent | Mean ± S.D. (No. of observations) | | *P* value | Significance |
|---|---|---|---|---|
| | Normal | Diabetic | | |
| Blood sugar (mgm./100 ml.) | 54·4 ± 35·1 (9) | 64·3 ± 37·5 (11) | > 0·5 | No |
| Total protein (gm./100 ml.) | 3·99 ± 0·27 (9) | 3·44 ± 0·23 (11) | < 0·01 | Yes |
| Albumin (gm./100 ml.) | 2·32 ± 0·28 (8) | 1·84 ± 0·28 (8) | < 0·01 | Yes |
| Cholesterol (mgm./100 ml.) | 134 ± 31 (8) | 101 ± 20 (10) | < 0·02 | Yes |
| Protein-bound hexose (mgm./100 ml.) | 53·5 ± 5·0 (8) | 43·4 ± 4·4 (6) | < 0·01 | Yes |
| Hæmatocrit, (per cent form of elements) | 46·4 ± 5·8 (13) | 51·0 ± 6·7 (23) | < 0·05 | Yes* |

* Significant at a 5 per cent level only after enlarging the series of animals tested.

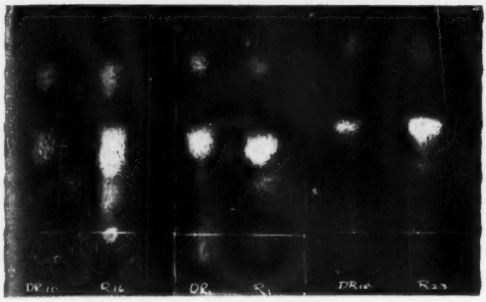


Fig. 1. Electrophoretic patterns of plasma proteins stained for carbohydrate. $R_x$, Offspring of a normal rabbit; $DR_x$, offspring of a diabetic rabbit

Fig. 1 is a photograph of a few representative plasma protein patterns stained for carbohydrate. It can be seen that the amounts of stainable material were diminished in all fractions from new-borns of diabetic females.

The low concentration of certain blood constituents and the low body-weights are presumably reflexions of the pathological maternal environment to which these animals were exposed during the foetal life. Further work is in progress.

Otakar V. Sirek
Anna Sirek

Department of Physiology and
Banting and Best Department of Medical Research,
University of Toronto.

[1] King, E. J., "Micro-Analysis in Medical Biochemistry", 23, 2nd edit. (J. and A. Churchill, London, 1951).
[2] Bloor, W. R., Pelkan, K. F., and Allen, D. M., *J. Biol. Chem.*, **52**, 191 (1922).
[3] Lustig, B., and Langer, A., *Biochem. Z.*, **242**, 320 (1931).
[4] Weichselbaum, T. E., *Amer. J. Clin. Path., Tech. Sect.*, **16**, 40 (1946).
[5] Kunkel, H. G., "Methods of Biochemical Analysis", **1**, 144 (1954).
[6] Björnsjö, K. B., *Scand. J. Clin. and Lab. Invest.*, **7**, 153 (1955).
[7] Miller, H. C., *Endocrinol.*, **40**, 251 (1947).

## Saliva-Serum Ratios of Deuterium Oxide after Administration of Heavy Water

Total body water may be estimated by administering a known weight of deuterium oxide and, after equilibration has occurred, determining its concentration in water obtained from some body fluid. Faller *et al.*[1] have shown that, within the limits of experimental error, the same values are obtained for total body water whether the deuterium oxide is administered orally or intravenously. Serum is the usual source of body water containing the equilibrium concentration of deutrium oxide. If, however, urine or saliva could be used, the whole procedure could be carried out without venipuncture; this would be an advantage when serial estimations are to be made or when other investigations involving venipuncture are undertaken in the same subject.

Urine formed during the period 3—6 hr. after administration of deuterium oxide yields water with a deuterium oxide concentration equal to that of a serum sample obtained during the same period[2,3]. Unfortunately the method of Schloerb *et al.*[4] for estimation of deuterium oxide cannot be applied directly to urine, which contains 'oxidisable impurities' that are not removed by simple vacuum distillation. The modified distillation procedure of Faller *et al.*[2] enables samples of urine instead of serum to be used, but is longer and more laborious.

Within about 2 hr. after administration, deuterium oxide is distributed uniformly throughout many body fluids, including arterial and venous blood, urine, liver water, gastric juice and cisternal fluid[5]. No report comparing salivary concentrations of deuterium oxide with those in blood has been found. Tritium apparently equilibrates in body water so that its concentration is similar in serum, urine, saliva, sweat, faeces and insensible perspiration[6] within the limits of sensitivity of the method used; the concentrations of tritium involved are extremely low.

Table 1

| Subject | Weeks of gestation | Time-interval (hr.) between $D_2O$ admin., and removal of samples | Serum conc., gm. $D_2O$/100 ml. | Saliva conc. / Serum conc. |
|---|---|---|---|---|
| A. | 8 | 3 | 0·154 | 1·05 |
| | 24 | 3 | 0·218 | 1·05 |
| | 30 | 3 | 0·211 | 1·22 |
| B. | 12 | 3 | 0·136 | 1·14 |
| | 36 | 6 | 0·149 | 1·23 |
| | 36 | 24 | 0·136 | 1·39 |
| C. | 11 | 3 | 0·144 | 1·20 |
| D. | 12 | 3 | 0·134 | 1·17 |
| H. | 14 | 3 | 0·157 | 1·12 |
| K. | 22 | 3 | 0·175 | 1·09 |
| | 31 | 3 | 0·226 | 1·09 |
| M. | 11 | 3 | 0·161 | 1·34 |
| P. | 31 | 3 | 0·152 | 1·26 |
| | 10 days post-partum | 3 | 0·230 | 1·37 |
| | 2 weeks post-partum | 3 | 0·250 | 1·20 |
| S. | 21 | 3 | 0·140 | 1·12 |
| Sp. | 15 | 3 | 0·150 | 1·26 |
| | | | | Mean: 1·19 |

The subjects were normal pregnant women. Sufficient deuterium oxide was given, orally, to bring the equilibrium serum concentration within the range 0·100 to 0·250 gm. deuterium oxide per 100 ml. of serum water. Immediately afterwards, patients had breakfast. Three hours after administration of deuterium oxide, and approximately 2½ hr. after breakfast, samples of venous blood and of saliva were taken. For the saliva sample, the women were encouraged to salivate and swallow the saliva for a few minutes before providing the sample, thus ensuring that it was recent secretion. Water was obtained from the serum and saliva by double vacuum distillation and the deuterium oxide concentration in the water was estimated by a modification of the falling-drop method described by Schloerb *et al.*[4].

In one subject samples of blood and of saliva were taken 6 hr. and 24 hr. after administration of deuterium oxide. In all, 17 pairs of estimations were made.

Table 1 shows the ratios of saliva concentration to serum concentration of deuterium oxide, together with the corresponding serum concentrations. The ratios are all greater than unity, the mean being 1·19 and the range 1·05—1·39. Absolute serum concentration of deuterium oxide has no effect on the ratio, but two women, A. and K., tend to have ratios only slightly above unity.

Although the possibility exists that some substance in saliva distils across with the water and

affects the density of the drops, there is no reason to suspect this, and the results indicate that the salivary glands concentrate deuterium oxide above the level in serum, at least at the low levels of concentration in these experiments. Salivary gland appears to be unique among human tissues so far investigated in this ability.

Nan Taggart
F. E. Hytten

Obstetric Medicine Research Unit
(Medical Research Council),
Aberdeen Maternity Hospital,

[1] Faller, I. L., Petty, D., Last, J. H., Pascale, L. R., and Bond, E. E., *J. Lab. Clin. Med.*, **45**, 748 (1955).
[2] Faller, I. L., Bond, E. E., Petty, D., and Pascale, L. R., *J. Lab. Clin. Med.*, **45**, 759 (1955).
[3] Hurst, W. W., Schemm, F. R., and Vogel, W. C., *J. Lab. Clin. Med.*, **39**, 41 (1952)
[4] Schloerb, P. R., Friis-Hansen, B. J., Edelman, I. S., Sheldon, B. B., and Moore, F. D., *J. Lab. Clin. Med.*, **37**, 653 (1951).
[5] Edelman, I. S., *Amer. J. Physiol.*, **171**, 279 (1952).
[6] Pinson, E. A., *Physiol. Rec.*, **32**, 123 (1952).

## Effect of Cell-Free Extracts from *Mycobacterium tuberculosis* $H_{37}Rv$ on Lung Succinooxidase

Segal and Bloch showed that non-proliferating suspensions of human tubercle bacilli grown *in vivo* (*LRv*) exhibited different biochemical properties as compared with the same strain of tubercle bacilli grown *in vitro*: (*a*) they had a lower hydrogen transfer capacity; (*b*) glucose and its intermediates failed to cause an increase over their endogenous respiration[1].

The experiments reported here were concerned with the electron transfer capacity of cell-free extracts from tubercle bacilli grown *in vivo* and *in vitro* and with the effect of these extracts on the respiration of lung homogenates.

The bacilli grown *in vivo* were obtained from the lungs of moribund or dead mice infected intravenously with the human strain of *M. tuberculosis* $H_{37}Rv$. To obtain a good yield each mouse was given intramuscular injections of 1·2 mgm. cortisone acetate every second day starting from the fourteenth day after infection. The bacilli were isolated from the lungs by the method employed by Segal and Bloch[1]. The *in vitro* grown tubercle bacilli strains $H_{37}Rv$ and *BCG* were obtained from 10–20 days cultures on 'Tween' albumin medium. The cells were separated from the culture media by centrifugation and washed twice in 0·1 *M* phosphate buffer *p*H 7·1. Cell-free extracts were obtained by disrupting of the cells in a 9 *KC* Raytheon sonic oscillator for 30 min. and the debris removed by centrifugation at 9,000 r.p.m. for 10 min. at 5° C.

The hydrogen transfer capacity of the extracts was examined by the reduction of triphenyltetrazolium chloride in the presence of different substrates. The cell-free extracts of *BCG* and $H_{37}Rv$ reduce tetrazolium in the presence of lactate, malate and succinate whereas the cell free extracts of $H_{37}Rv$ grown *in vivo* ($LH_{37}Rv$) did not show any activity in this respect. Since it was difficult to believe that $LH_{37}Rv$ extracts would be entirely devoid of hydrogen transfer capacity, the assumption was tested that their inactivity was due to the presence of an inhibitor. $LH_{37}Rv$ extracts were incubated with active *BCG* preparations in the presence of lactate. As seen from the experiment summarized in Table 1, this assumption proved to be correct. The cell-free extracts from $LH_{37}Rv$ inhibited the lactic dehydrogenase of *BCG* extracts from 50 up to 100 per cent.

Table 1. Inhibition of Lactic Dehydrogenase of *BCG* Cell-Free Extracts by Cell-Free Extracts of $LH_{37}Rv$

| | O.D. of formazan formed in the presence of lactate by the extracts from | | | Inhibition (per cent) |
|---|---|---|---|---|
| | $LH_{37}Rv$ | *BCG* | *BCG* + $LH_{37}Rv$ | |
| Exp. 1 .. | 0·00 | 2·44 | 1·22 | 50 |
| Exp. 2 .. | 0·00 | 3·20 | 0·00 | 100 |
| Exp. 3 .. | 0·00 | 2·20 | 0·24 | 89 |

System: Cell-free extracts (equivalent to 6·5 mgm. protein) 0·5 ml. in 0·1*M* phosphate buffer *p*H 7·1; lactate, 0·3 *M*, 0·3 ml.; 1 per cent solution of triphenyltetrazolium chloride, 0·2 ml. Time of incubation 1 hr. Temperature, 37° C. The formazan was extracted with *iso*-butanol and read at 485 mμ in a Coleman Jr. spectrophotometer.

This result prompted us to test the action of $LH_{37}Rv$ extracts on normal lung tissue homogenates. For this purpose lungs of normal mice were homogenized in 0·25*M* sucrose and their oxygen uptake was measured by the conventional Warburg method in the presence of the extracts. Table 2 shows that the extracts of $LH_{37}Rv$ inhibited the succinooxidase of lung tissue from 31·1 up to 53 per cent. The extracts of $H_{37}Rv$ and *BCG* were without any effect except for one experiment in which $H_{37}Rv$ extract inhibited the oxidation of succinate by 14 per cent.

Table 2. Effect of Mycobacterial Extracts on Succinooxidase of Normal Mice Lung Homogenates

| Source of enzyme | μl oxygen per hour* | | | |
|---|---|---|---|---|
| | Exp. 1 | Exp. 2 | Exp. 3 | Exp. 4 |
| Lung homogenate | 27·2 | 50·4 | 33·0 | 36·1 |
| *BCG* + Lung homogenate | 27·2 | 53·2 | 33·0 | not examined |
| $H_{37}Rv$ + Lung homogenate | 27·3 | 43·2 | 34·3 | not examined |
| $LH_{37}Rv$ + Lung homogenate | 15·2 | 31·1 | 15·5 | 20·0 |

The Warburg vessel contained: 10 per cent suspension of lung homogenate in 0·25 *M* sucrose, 0·4 ml.; where present, bacterial extracts, 0·5 ml.; 0·3 *M* succinate, 0·3 ml. tipped from side arm after 15 min. equilibration; phosphate buffer 0·1 *M*, 0·5 ml. (*p*H 7·1); 15 per cent solution of potassium hydroxide, 0·2 ml. in centre well. The final volume 2·2 ml. Temperature 37° C.
* The values are corrected for oxygen uptake off bacterial extracts.

A detailed report will be given later.

A. Bekierkunst
M. Artman

Department of Bacteriology,
Hebrew University,
Hadassah Medical School,
Jerusalem.
June 7.

[1] Segal, W., and Bloch, H., *J. Bacteriol.*, **72**, 132 (1956).

## Seasonal Changes in the Œstrous Response by the Ovariectomized Ewe to Progesterone and Œstrogen

Robinson *et al.*[1] have presented results of the quantitative requirements of progesterone and œstrogen for œstrous behaviour in the spayed Merino cross-bred ewe. These results were derived mainly from experiments conducted over short periods (2–3 months) and within the normal œstrous season of that ewe. We have noted[2] that the œstrous response of the spayed Romney ewe following progesterone-œstrogen treatment during November and December (months within the anœstrous season) was less than during the œstrous season. This difference could have resulted from the vasectomized rams exhibiting reduced libido during the summer and so failing to mark all ewes in œstrous[3]. However, our observations suggest that rams in this district will detect ewes in œstrous as we have seen following treatment of ewes

with progesterone and pregnant mares' serum during the anœstrous season. Also, ewes have been marked at this time following administration of greater quantities of œstrogen, than previously[2]. Therefore we have investigated further the response to progesterone and œstrogen in the spayed ewe.

Trials were conducted with spayed Romney ewes during January 1957–May 1959. The 12 spayed ewes previously mentioned[2] were used during January–July 1957. For the remaining trials these ewes and an additional 8 spayed animals of similar age and breeding were treated. In each trial three groups of ewes were injected for three consecutive days with progesterone (10 mgm./day) followed 40 hr. later by œstradiol benzoate. The groups of ewes in a trial received either 10, 20 or 40 μgm. or 20, 40 or 80 μgm. œstradiol benzoate. The trials were conducted at 14-day intervals, but after each three consecutive trials a 3–6 week control period was allowed to elapse before further hormone treatment. The animals were run as one flock with one or two vasectomized rams and observations were made for mating marks. Mucus from the cervix was also collected on the three days following treatment with œstradiol benzoate and examined for crystallization patterns[4]. As well, random collections were made during the control periods and on some days of progesterone treatment.

Œstrus did not occur after an injection with 10 μgm. œstradiol benzoate. The œstrous response following injection with the higher quantities of œstrogen is given in Fig. 1. We noted previously[2] that similar progesterone treatment and 40 μgm. œstradiol benzoate resulted in one ewe of twelve treated being marked by the ram during November–December, 1956. We have again found this to be the case during the two following anœstrous seasons. However, during the œstrous season this same treatment has caused a greater œstrous response. A similar seasonal relationship appears to exist after treatment with 80 μgm. œstradiol benzoate.

Crystallization patterns in cervical mucus were found in practically all ewes treated. Even after the lowest levels of œstrogen (that is, 10 and 20 μgm. œstradiol benzoate) the response of cervical mucus has been similar throughout the experiment. In contrast cervical mucus collected and examined during the control periods has shown in most cases little or no characteristic crystallization patterns. Thus although in some of the target organs associated with œstrous behaviour the sensitivity to progesterone and œstrogen may be reduced during anœstrus, the response of cervical mucus appears unaffected by seasonal changes.

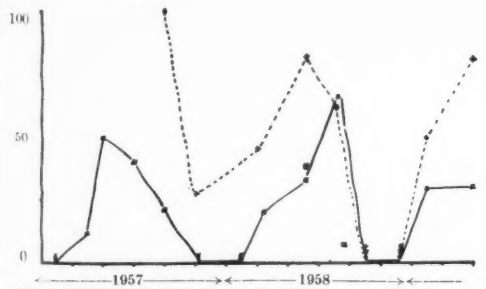



Fig. 1. Œstrous response in ovariectomized ewes following progesterone-œstrogen treatment.
Each point represents the percentage of ewes in œstrous for a series of three trials and plotted at the mean date for each series. Values obtained during December, 1958 represent one trial only
+ --- +, 80 μgm.; × —— ×, 40 μgm.; □, 20 μgm. ODB.

This work has been assisted by a financial grant from New Zealand Wool Board.

J. I. RAESIDE
M. F. MCDONALD

Sheep Husbandry Department,
Massey Agricultural College,
Palmerston North,
New Zealand.

[1] Robinson, T. J., *Nature*, **173**, 878 (1954); *Endocrinol.*, **55**, 403 (1954); *J. Endocrin.*, **12**, 163 (1955). Robinson, T. J., and Moore, N. W., *J. Endocrin.*, **14**, 97 (1956). Robinson, T. J., Moore, N. W., and Binet, F. E., *J. Endocrin.*, **14**, 1 (1956).
[2] McDonald, M. F., and Raeside, J. I., *J. Endocrin.*, **18** (in the press).
[3] McKenzie, F. F., and Berliner, V., Univ. Missouri Res. Bull. No. 265.
[4] Raeside, J. I., and McDonald, M. F., *J. Endocrin.*, **18** (in the press).

## Uræmia and its Treatment by Arginase Inhibitor

URÆMIA is retention of urea in the body, and is due to renal insufficiency. It has been proved beyond doubt that the kidney is the only organ through which urea can be eliminated from the body[1]. Therefore renal insufficiency of an organic or a functional type will reverse the clearance of urea from the body, with consequent retention. The methods involving intestinal and peritoneal dialysis, artificial kidney and possibilities of a successful kidney homotransplant for the treatment of uræmia are mainly based on the principle of eliminating urea from the body. The problem becomes very much simplified if technique can be evolved checking the synthesis of urea, which obviously could cause least damage to the patient.

In order to explore this possibility, experiments were performed involving the application of L-lysine monohydrochloride, the most powerful arginase inhibitor on dogs in an artificially created state of asthenic uræmia. It may be noted that arginase activity is essential in the synthesis of urea according to the classical work of Krebs[2]. The results so far noted are so consistently encouraging that an immediate announcement has been thought desirable in the interests of the medical practitioner in general and urologists in particular.

In spite of the fact that a number of arginase inhibitors[3,4] such as quinones, buffers, different amino-acids and some protein denaturants are known, L-lysine monohydrochloride was chosen in order to eliminate the different factors exerting harmful influence on the patients.

Control dogs (average weight of 10 kgm.) were induced to a state of asthenic uræmia by bilateral nephrectomy under a general anæsthetic. The level of blood urea (N) was recorded before and every 24 hr. after the operation. The urea (N) percentage was found to increase at an average rate of 15–16 mgm. every 24 hr. The animals died 80–84 hr. after operation at urea (N) level of 67–70 mgm. due to uræmia. During the period following operation, the animals were kept on a protein-free diet and received 50 gm. of glucose in 25 per cent solution intravenously daily in order to meet the basic calorie requirements and prevention of endogenous protein breakdown.

In another set of animals operations were performed. In this set, however, a dose of 1 gm. of L-lysine monohydrochloride in 10 per cent solution was injected intravenously daily after the operation. It was noted that the urea (N) percentage in the blood was found to increase only at the rate of 3–4 mgm. every 24 hr. in strong contrast to the control where it was 15–16 mgm. during the same period. The animals survived for 274–278 hr. after the operation at a urea (N) level of 68–70 mgm. per cent at the time

of death, whereas in no case in the control preparations the animals survived more than 84 hr.

In order to find out whether the survival period can be prolonged with an increased dose of L-lysine monohydrochloride in another set of experiments 1·5 gm. of L-lysine monohydrochloride in 10 per cent solution was injected intravenously daily. The result was found to be similar to the previous one. This indicates that activity-level of L-lysine monohydrochloride in inhibiting synthesis of urea reaches its optimum with a dose of only 1 gm. per every 24 hr.

It is worth pointing out that in normal animals, where bilateral nephrectomy was not performed, injections of L-lysine monohydrochloride no doubt caused a fall in the urea-level of the blood, but in all cases at least a level of 18 mgm. per cent of urea (N) was maintained in the blood indicating thereby that a minimum level of urea in the blood is always kept by normal animals, if necessary through extra-hepatic urea formation[5]. The latter process can also explain the steady and slight rise in urea-level in bilaterally nephrectomized animals treated with L-lysine monohydrochloride.

It is obvious that if urea synthesis can be minimized in a state of asthenic uræmia, the results of treatment with L-lysine monohydrochloride are expected to be very satisfactory when the kidneys are functioning at least partially.

I acknowledge with thanks the help received from the authorities of the Bengal Veterinary College, Calcutta and specially Prof. P. C. Sen Gupta for allowing me to work in their laboratory. I am also greatful to Emsons Pharmaceuticals Ltd. for the supply of L-lysine monohydrochloride.

Dhruba K. Sen

Nilratan Sircar Medical College and Hospital, Calcutta.

[1] Mann *et al.*, *Amer. J. Physiol.*, **69**, 382 (1924).
[2] H. A. Krebs, "The Enzymes", **2**, 866 (1951).
[3] D. M. Greenberg, "The Enzymes", **1**, 893 (1951).
[4] Hunter, A. and C. E. Downs, *J. Biol. Chem.*, **157**, 427 (1945).
[5] Bach, S. J., E. M. Crook, and S. Williamson, *Biochem. J.*, **38**, 325 (1944).

## Enhanced Synaptic Function due to Excess Use

The aim of the present investigation has been to give, in cats, excess use to the synapses of some monosynaptic pathways through the spinal cord, keeping other monosynaptic pathways as controls. In an initial aseptic operation the nerves to some muscles of a synergic extensor group in one hind-limb have been severed and capped to prevent regeneration. Since the remaining muscles of such a synergic group have to substitute for the whole group in supporting the weight of the animal, it can be expected that there will be an excess of stress on them; consequently there will be an increased discharge from their stretch receptors along the group I*a* afferent fibres, so giving an increased activation of the excitatory synapses on their motoneurones.

The nerves to the medial gastrocnemius, plantaris, tibialis posterior and flexor hallucis longus were severed and capped, leaving intact only the nerves to the lateral gastrocnemius, and flexor digitorum longus from the two groups of synergic extensor muscles. A dummy operation identical in all respects except for cutting and capping the nerves was performed on the other leg. The reflex pathways used as controls were from the biceps semitendinosus nerve and the deep peroneal nerve in both hind-limbs. They are particularly suitable for controls because they give large monosynaptic reflexes, and, since they subserve flexor muscles, they should be little affected by the extensor asymmetry induced in the hind-limbs by the operation.

After recovery for a few days the animals were exercised in a treadmill for 40 min. daily over 3–4 weeks, and appeared to spare the affected side very slightly, although compensating to some degree by everting that foot at the time of stepping off. At the final acute experiment under nembutal anæsthesia, all test and control nerves in both hind-limbs were prepared and placed on stimulating electrodes. The central cut ends of ventral roots *S*1, *L*7 and *L*6 on both sides were used for monophasic recording. Both legs and the back were made into pools of paraffin maintained at a constant temperature of 36–37° C. The stimulus strengths of the testing volleys at a frequency of 0·5 per sec. were always at least twice maximal for activation of the group I*a* afferent fibres, and the standard repetitive (tetanic) stimulation employed for post-tetanic conditioning in all experiments was 400 per sec. for 15 sec. Corresponding reflexes were investigated in quick succession on the two sides in order to eliminate temporal inequalities of temperature or depth of anæsthesia.

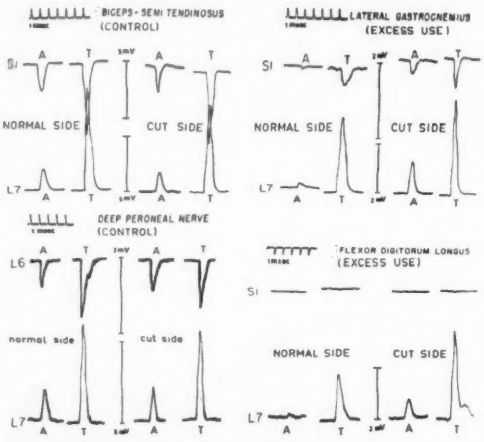



Fig. 1. All reflexes in this figure were taken from one typical experiment, in which the monosynaptic reflexes for each nerve were displayed simultaneously from two ventral roots. Reflex heights *A*, before post tetanic potentiation; *T*, after post tetanic potentiation are shown for each nerve tested in both limbs

On the left side of Fig. 1 the reflexes evoked by the biceps semitendinosus and the deep peroneal volleys before and after post-tetanic potentiation indicate a symmetry of monosynaptic reflexes into both *L*7 and *S*1 ventral roots. In contrast on the right side of Fig. 1 there is asymmetry for all monosynaptic reflexes evoked by lateral gastrocnemius and flexor digitorum longus volleys; invariably the reflexes were larger for the side on which these muscles were presumed to have been subjected to a prolonged excess stress on account of the denervation of their synergists. In each of 14 cats, up to four series of reflexes before and after post-tetanic potentiation were recorded with intervals of 1–2 hr. between series. There were slight increases, not statistically significant, for biceps semitendinosus and deep peroneal reflexes on the cut side, but marked increases, which averaged more than 50 per cent, in the reflexes from the lateral gastrocnemius and

flexor digitorum longus nerves when compared to the corresponding reflexes from the control side. These increases are statistically significant $P < 0.001$ except lateral gastrocnemius into S1VR for which $P < 0.05$. There were no changes of statistical significance in the conduction velocity of the afferent fibres or the absolute refractory periods for any nerve on the two sides; nor were there any significant differences between the fibre counts or distribution of fibre diameters for the lateral gastrocnemius and flexor digitorum longus nerves on the two sides. Muscle wet weights did not show any significant differences between the two sides, except a wasting of approximately 40 per cent in the denervated muscles.

In the absence of demonstrable changes in the afferent fibres we assume that the site of changes in excitability leading to the increased lateral gastrocnemius and flexor digitorum longus monosynaptic reflexes was either at the presynaptic terminals of the group I*a* fibres or the post-synaptic motoneurone membrane, that is, at the excitatory synapses. Possible mechanisms by which the presumed excess use could render synaptic action more efficient include increased size of presynaptic terminals or alterations in the numbers and/or disposition of the synaptic vesicles at the presynaptic terminals. With synaptic potency thus increased, more motoneurones in the pool would be activated by each testing volley, the monosynaptic reflex being correspondingly greater than on the control side. Past evidence that prolonged disuse adversely affects synaptic function,[1,2] together with this new evidence that excess use leads to an enduring increase of synaptic efficacy, provide strong support for the postulate that learning and conditioning are due to the enhancement of synaptic efficacy by excess use.

ROSAMOND M. ECCLES
R. A. WESTERMAN

Department of Physiology,
Australian National University,
Canberra.
June 26.

[1] Eccles, J. C., Krnjevic, K. and Miledi, R., *J. of Physiol.*, **145**, 204 (1959).
[2] Eccles, J. C., and McIntyre, A. K. *J. Physiol.*, **121**, 492 (1953).

## Effect of Sulphanilamide on Citric Acid Production by *Aspergillus niger*

IT has been shown that the mechanism of resistance to sulphanilamide toxicity in *E. coli* involves the enhanced formation of coenzyme *A* which is required for the acetylation of the drug[1]. A similar increase in the coenzyme *A* levels of the cells has been observed in *S. cerevisiae* subjected to sulphanilamide toxicity[2]. An interesting observation made in the experiments with *S. cerevisiae* was that, concomitant with the increase in the coenzyme *A* levels of the cells, there was an enhancement in the levels of ergosterol as well[2]. In view of the known role of coenzyme *A* in sterol biosynthesis, it was considered of interest to investigate the effect of sulphanilamide toxicity on other biosynthetic processes involving the action of coenzyme *A*. Accordingly the effect of the drug on citric acid production by a citric acid-accumulating strain of *Aspergillus niger* was investigated. The results presented here show that the production of citric acid by the mould is markedly inhibited by sulphanilamide.

The basal medium used in these studies was: glucose, 140 gm., ammonium nitrate, 2·5 gm., potassium dihydrogen phosphate, 2·5 gm. crystalline magnesium sulphate, 0·25 gm. crystalline manganese sulphate, 100 mgm. crystalline zinc sulphate, 1·25 mgm. distilled water to 1,000 ml. The *p*H of the medium was adjusted to 2·0–2·5 in all cases. All experiments were carried out in 250-ml. Erlenmeyer flasks containing 25 ml. culture medium.

The effect of sulphanilamide on accumulation of citric acid by growing cultures of *A. niger* as well as by resting mycelial pads was investigated. In the former case, sulphanilamide was introduced into the culture medium of growing cells of the organism and the incubation continued for a further specified period. The contents of each flask were analysed for dry weight of mycelia, total acids produced, citric acid formed and the amount of sugar consumed. Total acids were determined by titration of an aliquot of culture medium against 0·1 *N* sodium hydroxide. A colorimetric method[3] was employed for citric acid determination while reducing sugar determinations were carried out by a method involving the use of Somogyi's high alkali copper sulphate reagent[4]. The results are given in Table 1.

Table 1. EFFECT OF SULPHANILAMIDE ON GROWING CULTURES OF *Aspergillus niger*

| Period of incubation after addition (days) | Sulphanilamide added (mgm. per flask) | Dry weight of mycelia (mgm.) | Total acids produced (ml. of 0·1*N*) | Glucose consumed (*A*) (mgm./100 mgm. dry mycelium) | Citric acid formed (*B*) (mgm./100 mgm. dry mycelium) | Per cent molar yield ($B/A \times 100$) |
|---|---|---|---|---|---|---|
| 3 | 0 | 144 | 54·6 | 1305 | 179·0 | 11·39 |
| | 50 | 101 | 9·2 | 1439 | 33·4 | 1·99 |
| | 75 | 108 | 8·0 | 1409 | 32·8 | 1·99 |
| 6 | 0 | 183 | 91·0 | 1279 | 254·0 | 17·02 |
| | 50 | 112 | 20·5 | 1690 | 52·1 | 2·68 |
| | 75 | 103 | 11·3 | 1522 | 36·1 | 2·03 |
| | 100 | 109 | 8·2 | 1566 | 16·9 | 0·93 |

The organism was initially grown for 48 hr. in 15 ml. medium at a concentration equivalent to 25 ml. single strength medium. 10 ml. of sterile distilled water was then added to each of the control flasks, and 10 ml. sulphanilamide solution in appropriate concentration to the remaining flasks under aseptic conditions. Incubation was continued for 3 or 6 days, after which the flasks were sterilized and the content analysed. All values represent averages of duplicate determinations.

In experiments with resting mycelia of *Aspergillus niger* the replacement technique of Chugtai and Walker[5] was adopted. The organism was initially grown for 4 days to form strong and integrated pads of mycelia. The culture medium was then withdrawn and replaced aseptically by an equal volume of sterile basal medium devoid of glucose. After incubation overnight the medium was again withdrawn and replaced with sterile 10 per cent glucose solution (w/v) containing added sulphanilamide where required. The incubation was continued for a further period of 3 or 6 days and then the contents of each flask analysed as before. The results are given in Table 2.

Table 2. EFFECT OF SULPHANILAMIDE ON CITRIC ACID FORMATION BY RESTING MYCELIA OF *Aspergillus niger*

| Sulphanilamide added (mgm. per flask | Weight of mycelia (mgm. dry weight) | Glucose used up (*A*) (mgm./100 mgm. dry mycelium) | Citric acid formed (*B*) (mgm./100 mgm. dry mycelium) | Percent molar yield ($B/A \times 100$) |
|---|---|---|---|---|
| 0 | 245 | 480 | 98·0 | 17·5 |
| 25 | 235 | 454 | 31·9 | 6·0 |
| 50 | 222 | 456 | 22·9 | 4·4 |
| 100 | 240 | 483 | 5·6 | 1·0 |
| 125 | 250 | 516 | 5·4 | 0·9 |

Mycelial pads of the organism were incubated aseptically under resting conditions in 25-ml. lots of 10 per cent glucose solution, with addition of sulphanilamide as specified. The contents of each flask were analysed after 6 days' incubation in the replacement medium. All values represent averages of duplicate determinations.

It is seen from the results given in Tables 1 and 2 that sulphanilamide produces a profound inhibitory effect on the production of citric acid by *A. niger* under growing, as well as resting, conditions. That this inhibition is not caused by an impairment in carbohydrate utilization is obvious from the fact that there is no decrease in the relative amounts of glucose consumed. In another set of experiments it has been found that the addition of *p*-aminobenzoic acid to the medium reverses the effect of the drug on the final weight of the mycelia under growing conditions but does not have any effect on citric acid formation. Indeed, it can be expected that the vitamin will have the same effect on the acetylating system as sulphanilamide and hence it is not surprising that it does not antagonize the effect of the drug on citric acid formation. Again, the inclusion in the medium of a mixture containing adenine, guanine and uracil and the amino-acids methionine and serine, compounds the biosynthesis of which involves the action of *p*-aminobenzoic acid, reverses the inhibition of growth but not the inhibitory effect on citric acid production.

It would seem that the inhibitory effect of sulphanilamide on formation of citric acid in *A. niger* is the result of the stress produced by the drug on the acetylase system. The present results, therefore, emphasize the importance of acetate in citric acid synthesis by the mould.

K. V. Rajagopalan
R. Radhakrishnamurthy*
P. S. Sarma

University Biochemical Laboratory
Madras, 25. June 2.

* Present address: Biochemistry Division, Central Food Technological Research Institute, Mysore, 2.

[1] Kayser, F., and Metzinger, J., *C. R. Soc. Biol.*, **147**, 1460 (1954).
[2] Rajagopalan, K. V., and Sarma, P. S., *Biochem. J.*, **69**, 53 (1958).
[3] Saffran, M., and Denstedt, U. F., *J. Biol. Chem.*, **175**, 849 (1948).
[4] Somogyi, M., *J. Biol. Chem.*, **119**, 741 (1937).
[5] Chugtoi, I. D., and Walker, T. K., *Biochem. J.*, **56**, 484 (1954).

## A Chemical Effect of Ethylene during the Storage of Peas

In recent work[1] the author and others found that the crude lipid extracted from raw peas held in the pods in frozen storage (– 17·8 °C.) for periods of time, consistently developed much larger peroxide values than that extracted from raw peas of the same variety, harvested from the same plots at the same time, which were vined previous to placing in storage.

It occurred to me that this difference might be caused by ethylene in the atmosphere inside the pods.

The present experiment was designed to test this hypothesis. The peas used in this work were harvested from the same plot at the same time, and were of the Perfected Freezer variety. Raw peas were carefully shelled by hand to avoid injury and were packed in glass bottles filled with glass lead-in and exit tubes so that the bottles could be flushed with an atmosphere of known composition. These tubes were so constructed so that they could easily be sealed with a flame as soon as the flushing with the controlled atmosphere was completed. The following atmospheres were used for this purpose:

Gas I, 5 per cent carbon dioxide, 3 per cent oxygen, 92 per cent nitrogen
Gas II, 5 per cent carbon dioxide, 3 per cent oxygen, 91·98 per cent nitrogen, 0·02 per cent ethylene

Controls were run with raw peas held in the pods. A 7-month storage period at — 17·8°C. was employed for the results presented here.

The crude lipid was extracted and the peroxide numbers were determined as previously reported[2,3] (Table 1).

Table 1. Peroxide Values* of Extracted Crude Lipid

| Storage conditions | Gas I | Gas II | Held in pods |
|---|---|---|---|
| Peroxide value | 5·6 | 19·1 | 20·4 |

* Millimoles of peroxide oxygen per kgm. of lipid; average of determinations in triplicate.

It seems, therefore, that ethylene has an action on the lipid matter of peas stored in the presence of this gas. The peas stored in the atmosphere containing ethylene yielded crude lipid which gave a high peroxide value. It is interesting to note that the peroxide value of the lipid extracted from the peas held in the controlled atmosphere containing ethylene was almost identical with that obtained from the lipid extracted from the peas stored in the pods.

Since it was found in previous work[1] that storage in the pods retards the deterioration in flavour, it may be suspected that ethylene and possibly other gases have a part in bringing about this result.

It is possible, therefore, that the lipid fraction, small as it is, may be involved in the biochemistry of the normal ripening of fruits and certain vegetables.

The results of this work will be published in detail elsewhere.

I acknowledge with thanks the technical assistance of Miss Kathleen Thomas.

Frank A. Lee
New York State Agricultural Experimental Station,
Cornell University,
Geneva, New York.

[1] Lee, F. A., Wagenknecht, A. C., and Graham, R., *Food Res.*, **21**, 666 (1956).
[2] Lee, F. A., *Food Res.*, **19**, 515 (1954).
[3] Lees, F. A., and Wagenknecht, A. C., *Food Res.*, **16**, 239 (1951).

## Isolation of Fatty Alcohols with Plant-Growth Promoting Activity from Maryland Mammoth Tobacco

It has been reported previously that 3-indoleacetic acid could not be detected in leaves or apical tissues of Maryland Mammoth tobacco[1]. Consequently, further attempts have been made to define more clearly the chemical factors responsible for the growth and development of this variety.

Leaves and apical tissues of two-month-old Maryland Mammoth tobacco plants were harvested, frozen rapidly in solid carbon dioxide, and ground in absolute ethanol. The subsequent extraction procedure was as described previously[1]. The final extracts were chromatographed on a Gryksbo chromatographic filter paper column (type *LKD*-3391) with a steady flow of solvent consisting of *iso*propanol: ammonium hydroxide:water (80:5:15 v/v/v). Successive 100-ml. fractions of percolate were removed at the bottom of the column until a total of three litres had been collected, and each fraction was stored at — 20° C.

A light-coloured, oily precipitate separated in fractions 25–28 after a few days. This material was collected by centrifugation and dried over calcium chloride. The dry, tan solid then was subjected to a process of fractional crystallization from absolute ether which afforded finally several mgm. of a waxy solid exhibiting growth-promoting activity in the bioassay mentioned below.

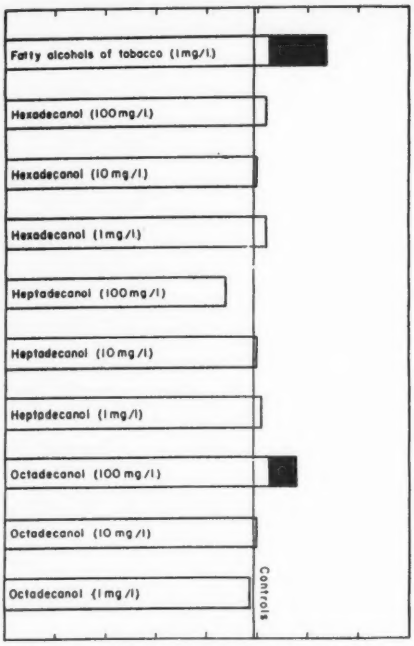



Fig. 1. Final mean lengths for ten *Avena* first-internode sections floated for 22 hr. in buffer (with sucrose) containing the fatty alcohols isolated from tobacco, hexadecanol, heptadecanol, and octadecanol. Shaded areas represent statistically significant differences ($P = 0{\cdot}01$)

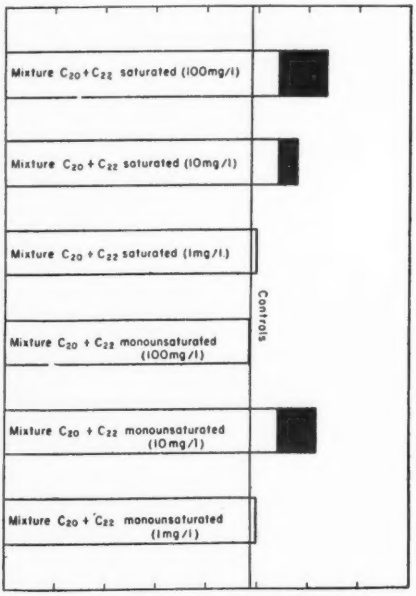



Fig. 2. Final mean lengths for ten *Avena* first-internode sections floated for 22 hr. in buffer (with sucrose) containing $C_{20}+C_{22}$ saturated alcohols, and $C_{20}+C_{22}$ unsaturated alcohols. Shaded areas represent statistically significant differences ($P = 0{\cdot}01$)

The white crystals were soluble in ether, ethanol, and benzene, but were insoluble in water. The solid melted at 70–72° C. on a Vanderkamp block, and resolidified only very slowly. Nitrogen, sulphur, halogens, and phosphorus were not present in its elementary composition. Even in concentrated hexane solution, no ultra-violet absorption was observed, indicating the absence of unsaturation. Infra-red spectra, measured on a Perkin-Elmer model 12*A* spectrophotometer equipped to handle micro-samples, were found to be almost identical with those of long-chain normal primary alcohols, such as 1-docosanol.

Nilsson, Ryhage, and von Sydow[2] have isolated a neutral, crystalline substance melting at 70–74° from air-dried pollen of *Pinus montana*. Mass-spectrometric investigation, using a special high-temperature instrument, revealed that their isolate was a mixture of 1-tetracosanol, 1-hexacosanol, and 1-octacosanol. Although our own mass-spectrometric evidence is inconclusive, it is quite likely that we, too, have isolated such a mixture.

The plant-growth regulating activity of the tobacco isolate, as well as that of sixty long-chain fatty alcohols and related compounds, was measured by means of the *Avena* first-internode bio-assay[3]. Results of typical experiments are shown in Figs. 1 and 2, in which shaded areas represent elongation which is statistically significant at the 1 per cent level. These values were reproduced on at least two subsequent occasions in each case. The details and results of further investigation will be discussed elsewhere in a future publication. However, in general, only $C_{18}$ to $C_{22}$ alcohols and their acidic esters were found to exhibit growth-regulating activity in this series.

The absence of 3-indoleacetic acid in tissues of Maryland Mammoth tobacco, coupled with the demonstration of growth-regulating activity in a molecule very different from the indole type, suggests that the present over-simplified concepts of the hormonal regulation of plant growth are in need of re-evaluation.

A. J. Vlitos*

Boyce Thompson Institute for Plant Research, Inc.,
Yonkers, New York.

D. G. Crosby

Union Carbide Chemicals Company,
Research Department,
South Charleston, W. Va.
June 8.

* Present address: Caroni Ltd., and Ste. Madelaine Sugar Co., Carapichaima, Trinidad.

[1] Vlitos, A. J., Meudt, W., and Beimler, R., *Nature*, **177**, 890 (1956).
[2] Nilsson, M., Ryhage, R., and von Sydow, E., *Acta Chem. Scand.*, **11**, 634 (1957).
[3] Nitsch, J. P., and Nitsch, C., *Plant Physiol.*, **31**, 94 (1956).

## Essential Pentosuria: Renal or Enzymic Disorder

Essential pentosuria is a rare, recessive genetic metabolic disorder characterized by the excretion of gram quantities of L-xylulose. It was included among Garrod's "inborn errors of metabolism"[1] and has usually been considered to be the result of an enzyme deficiency. Garrod's original concept of a hereditary metabolic anomaly has been broadened by some to include renal defects, and, in regard to essential pentosuria, Knox[2] has recently stated, "the paramount question of a renal or an enzymic mechanism is still to be decided".

Our experiments were based on the use of D-glucuronolactone to stimulate xylulose production in human subjects. Enklewitz and Lasker[3] had, in 1935, discovered this phenomenon in pentosurics by measuring the excretion of the pentose in urine, and Touster and his co-workers[4] more recently showed that

a small but definite effect is demonstrable in animals and in normal humans as well. Moreover, experiments using isotopes showed that D-glucuronolactone is a direct precursor of urinary L-xylulose in the pentosuric[5], and that the metabolism of glucuronolactone in the pentosuric is blocked at L-xylulose[6].

If a renal defect exists in pentosuria, the administration of D-glucuronolactone should not cause a greater increase in blood xylulose-levels of pentosurics than of normal individuals because the pentose would be so readily excreted by the pentosuric. If pentosuria is due to a metabolic (enzymic) defect, on the other hand, glucuronolactone should result in higher blood xylulose concentrations in pentosuric individuals than in normal persons. Flynn[7], using paper chromatography, found that glucuronolactone administration augments the trace of xylulose normally found in pentosuric plasma, but comparable experiments on normal individuals were not done.

Five gm. of D-glucuronolactone, dissolved in about 200 ml. of water, were taken orally in one dose by our subjects. This quantity is known to yield 1–2 gm. of additional urinary xylulose in pentosuric subjects[3,4]. At the times indicated in Table 1, blood samples were

Table 1. Effect of 5 gm. of Oral Glucuronolactone on the Plasma Xylulose-Levels of Normal and Pentosuric Subjects

| Subject | Plasma xylulose (mgm./100 ml.) | | |
|---|---|---|---|
| | Fasting | 1 hr. | 2 hr. |
| Normal (M.K.P.) | <0·3 | <0·3 | <0·3 |
| Normal (R.C.B.) | <0·3 | <0·3 | <0·3 |
| Pentosuric (I.B.) | <0·3* | 9 | 8 |
| Pentosuric (I.B.) | 2* | 7 | 11 |

* In two other samples of fasting pentosuric plasma, the xylulose-level was less than 0·3 mgm./100 ml.

obtained by venipuncture from each subject. The heparinized blood was centrifuged to obtain the plasma, which was deproteinized with 20 per cent trichloroacetic acid (5 per cent final concentration). The filtrate was extracted several times with diethyl ether to remove the trichloroacetic acid and was then freed of ionic compounds by means of a mixed bed resin of 'Amberlite *IR*-120($H^+$)'-'Amberlite *IR*-400 (acetate)' (3 gm. of mixed resin per 5 ml. of original plasma). The deionized solution was concentrated to dryness *in vacuo* at 45–55°, and the residue was dissolved in a small amount of absolute ethanol. After clarification by centrifugation, the solution was analysed by paper chromatography in 88 per cent phenol. Synthetic xylulose and pentosuric urine were used as standards. The values given in Table 1 are based on visual comparison of the plasma xylulose chromatographic spots with the standards, after spraying the paper strips with either naphthoresorcinol[8] or phloroglucinol[9] reagents. The use of several other sugars as standards showed clearly that the plasma pentose was xylulose. The lower limit of detection of the ketopentose was about 0·3 mgm. per 100 ml. of plasma.

All subjects were adult males in apparent good health. One (I.B.) exhibits typical findings of essential pentosuria. The two controls do not excrete readily detectable quantities of xylulose.

Table 1 clearly shows that the pentosuric plasma increases markedly in xylulose concentration after the administration of glucuronolactone, a result in full accordance with the concept of a metabolic defect. The magnitude of the increase is of the order expected from the known effect of glucuronolactone on urinary xylulose-levels in persons with the anomaly. The unavailability of our subject for further experiments made it impossible to establish the time of attainment of maximum blood xylulose concentration or to investigate the variation in the fasting xylulose-level in the pentosuric. The plasma xylulose of the controls remained below the level of detection, undoubtedly a result of a considerable capacity to utilize xylulose as soon as it is formed from the administered glucuronolactone.

The missing or deficient enzyme in essential pentosuria is presumably triphosphopyridine nucleotide-xylitol (L-xylulose) dehydrogenase, a very specific enzyme which has been found in the liver of several species[10–12]. This enzyme catalyses the reduction of L-xylulose to xylitol in the glucuronate-xylulose, or $C_6$ oxidation, pathway of carbohydrate metabolism[13,14]. The relationship of this pathway to the metabolism of xylulose in normal and pentosuric humans has been discussed in two recent reviews[2,15]. There is no evidence to support the suggestion that L-xylulose excretion by pentosuric individuals may be due to the presence of an abnormal enzyme which stimulates the production of the pentose[7,16]. The determination of the exact nature of the enzymatic abnormality in essential pentosuria will probably depend ultimately on a comparison of the concentration of the L-xylulose-xylitol enzyme in normal and pentosuric liver.

The glucuronolactone loading test described in this report not only appears to rule out the renal hypothesis for the pentosuric defect, but it may be of value in detecting heterozygotes carrying the pentosuric gene. This possibility is now being explored.

This work was supported by a research grant from the National Science Foundation.

R. C. Bozian*
Oscar Touster†

Vanderbilt University School of Medicine,
Nashville, Tennessee.

* Supported by a Graduate Training Grant from the U.S. Public Health Service.

† Investigator of the Howard Hughes Medical Institute.

[1] Garrod, A. E., *Lancet*, **2**, 214 (1908).
[2] Knox, W. E., *Amer. J. Human Genet.*, **10**, 385 (1958).
[3] Enklewitz, M., and Lasker, M., *J. Biol. Chem.*, **110**, 443 (1935).
[4] Touster, O., Hutcheson, R. M., and Rice, L., *J. Biol. Chem.*, **215**, 677 (1955).
[5] Touster, O., Mayberry, R. H., and McCormick, D. B., *Biochim. Biophys. Acta*, **25**, 196 (1957).
[6] Hiatt, H. H., *Biochim. Biophys. Acta*, **28**, 645 (1958).
[7] Flynn, F. V., *Brit. Med. J.*, **i**, 391 (1955).
[8] Bryson, J. L., and Mitchell, T. S., *Nature*, **167**, 864 (1951).
[9] Borenfreund, E., and Dische, Z., *Arch. Biochem. Biophys.*, **67**, 239 (1957).
[10] Touster, O., Reynolds, V. H., and Hutcheson, R. H., *J. Biol. Chem.*, **221**, 697 (1956).
[11] Hollmann, S., and Touster, O., *J. Biol. Chem.*, **225**, 87 (1957).
[12] Hickman, J., and Ashwell, G., *J. Biol. Chem.*, **234**, 758 (1959).
[13] Burns, J. J., and Kanfer, J., *J. Amer. Chem. Soc.*, **79**, 3604 (1957).
[14] McCormick, D. B., and Touster, O., *J. Biol. Chem.*, **229**, 451 (1957).
[15] Touster, O., *Amer. J. Med.*, **26**, 724 (1959)
[16] Everett, M. R., "Medical Biochemistry", 2nd ed. (Paul Hoeber, New York, 1946).

## RADIOBIOLOGY

### Free Radicals in X-Rayed Seeds of High and Low Water Content, as Measured by Electron Spin Resonance

Recently Caldecott[1,2] and Ehrenberg[3,4] have shown that contrary to earlier ideas dry stored seeds of barley are more sensitive to ionizing radiation than are normal stored seeds. These results have been confirmed by one of us[5] in seeds of *Vicia faba*.

In order to find some possible explanation for this remarkable effect we have studied the free-radical content of X-rayed *Vicia faba* seeds by means of electron spin resonance absorption. The material used was an inbred line of *Vicia faba* var. minor, Throws M.S. Winter Beans from Hasler and Co., Ltd., Dunmow, Essex. Measurements were carried out separately with embryos excluding the cotyledons

(that is, radicles and plumules), with pieces of cotyledons and with pieces of the testa, using normally stored material (52 per cent relative air humidity) and dry stored material (2·5 per cent relative air humidity, storage 4 days over calcium chloride at 20° C.). The water content of the material under these conditions of air humidity was measured and is shown in Table 1.

Table 1. Water Content of Different Parts of *Vicia* Seeds After Storage Under Normal or Under Dry Conditions

| Relative humidity of air (per cent) | 52 | 2·5 |
|---|---|---|
| Plumules and Radicles (per cent) | 9·6 | 4·6 |
| Cotyledons (per cent) | 12·2 | 4·6 |
| Testas (per cent) | 12·0 | 4·3 |

Doses of 10,000 r. X-rays were given at 250 kV., 15 m.amp., using 0·5 mm. copper + 1 mm. aluminium filter, intensity 500 r./min. The dry material was irradiated in micro-desiccators. Within a few minutes after irradiation samples were transferred to quartz tubes and cooled down in liquid oxygen (90° K.). The electron spin resonance spectrometer used for the radical detection operates at 10 kMc./s., the cavity being cooled by liquid oxygen (ref. 6, pp. 52–53). The material was introduced into the cavity in weighed amounts so that the whole volume was always within ½ cm. of the centre of the cavity. The free radical concentration was determined by a comparison of the integrated absorption obtained from the sample and that obtained from a standard carbon sample of known radical content. It is estimated that the absolute accuracy of such measurements is about 50 per cent.

We found no free radicals in normally stored unirradiated material or in normally stored irradiated embryos and cotyledons (sensitivity about $10^{15}$ radicals per gm. dry weight). However, free radicals are present in normally stored testas after irradiation (about $8 \times 10^{15}$ radicals per gm. dry weight). We found no free radicals in dry stored unirradiated embryos and cotyledons, but obtained a distinct signal from dry stored unirradiated testas, indicating about $8 \times 10^{15}$ free radicals per gm. dry weight. These were not, as might be suspected, induced by the freezing process (ref. 6, p. 241), as in dry testas an equal signal appears at room temperature. In dry stored irradiated material clear evidence of free radicals was found in all parts of the seed. The concentration in the testa had risen to about $2 \times 10^{16}$, concentrations of about $7 \times 10^{15}$ radicals per gm. dry weight being calculated for embryos and cotyledons.

These results indicate that there is some correlation between the X-ray sensitivity as measured by biological damage and the production or survival of radiation-induced free radicals in seeds. In dry seeds higher concentrations of free radicals are built up during irradiation and the biological damage is greater. It may well be that these free radicals play an important part in the sequence of events from the primary effects of the radiation to the ultimate observed biological damage. This suggestion is supported by the recent results of Ehrenberg *et al.*[7,8] from entire seeds of *Agrostis stolonifera*. Moreover certain after-effects in X-rayed dry stored seeds[9–12] may be attributable to the action of such radicals whose survival depends upon conditions of storage. It should be borne in mind, however, that relatively high concentrations of free radicals are induced by radiation in what might appear to be the less-vital parts of the seed namely the testa and, further, that dry storage itself appears to induce free radicals. Dry storage also produces biological damage in the seeds[5]. How closely these effects are connected is at present a matter for speculation but they are clearly of importance in the interpretation of further experiments.

We wish to express our sincere thanks to Dr. J. R. Clarkson of the Royal South Hants and Southampton Hospital for taking charge of the irradiation. One of us (W.K.) is indebted to the Department of Scientific and Industrial Research and the Deutsche Akademische Austauschdienst for a research studentship in Great Britain. Another (M.S.) acknowledges with thanks a research fellowship under the Colombo plan.

W. Klingmüller
G. R. Lane

Department of Botany,

M. C. Saxena
D. J. E. Ingram

Department of Electronics,
University of Southampton.
June 10.

[1] Caldecott, R. S., *Rad. Res.*, **2**, 339 (1955).
[2] Caldecott, R. S., *Rad. Res.*, **3**, 316 (1955).
[3] Ehrenberg, L., *Botaniska Notiser*, **108**, 184 (1955).
[4] Ehrenberg, L. *Hereditas*, **41**, 123 (1955).
[5] Klingmüller, W., *Z. f. Naturforschung*, **14**, *b*, 268 (1959).
[6] Ingram, D. J. E., "Free Radicals as Studied by Electron Spin Resonance" (Butterworth, London, 1958).
[7] Ehrenberg, A., and Ehrenberg, L., *Arkiv Fysik*, **14**, 133 (1958).
[8] Zimmer, K. G., *Rad. Res.*, Suppl., **1**, 519 (1959).
[9] Adams, J. D., Nilan, R. A., and Gunthardt, H. M., *Northwest Science*, **29**, 101 (1955).
[10] Adams, J. D., and Nilan, R. A., *Rad. Res.*, **8**, 111 (1958).
[11] Curtis, H. J., Delihas, N., Caldecott, R. S., and Konzak, C. F., *Rad. Res.*, **8**, 526 (1958).

## BIOLOGY

### Interaction of Plant Growth Regulators in Regeneration Processes

In previous years much attention has been paid to the influence of growth regulators on regeneration processes, especially to the interactions of kinetin and auxin in morphogenesis of tissue cultures[1]. In experiments with *Begonia* leaves we have observed a decisive effect of auxin and kinetin on regeneration. In addition it could be shown that gibberellin profoundly affects the complex hormone system apparently controlling differentiation in higher plants.

In the experiments leaf disks (12 mm. diam., cut so as to include a portion of a secondary vein) were used. After washing the leaves in 3 per cent hydrogen peroxide the disks were cut and kept for 60 hr. on an isotonic salts solution (*p*H 5·5) with or without addition of 2,4-dichlorophenoxyacetic acid, kinetin or gibberellin, or combinations of these. After this pretreatment the disks were placed in Petri dishes on filter paper moistened with tap water.

Under the experimental conditions the control disks developed after 2–3 weeks a root at the base of the longest vein, and after a further 2–3 days a shoot appeared at the same position. This pattern of regeneration, which so far has always been observed in these experiments (August 1958–July 1959), was shifted by treatment with 2,4-dichlorophenoxyacetic acid in favour of root formation. That is, with concentrations of this acid between $10^{-8}$ and $10^{-5}$ gm./ml., often 3 or 4 roots were produced, and these developed up to 8 days earlier than in the controls. At the lower concentrations (up to $10^{-7}$ gm./ml.) production of the shoot was delayed for only 8 days relative to the controls, but at the higher concentrations for at least 3–4 weeks or indefinitely.

Kinetin (6-furfurylaminopurine) had a twofold effect. It not only suppressed the formation of roots

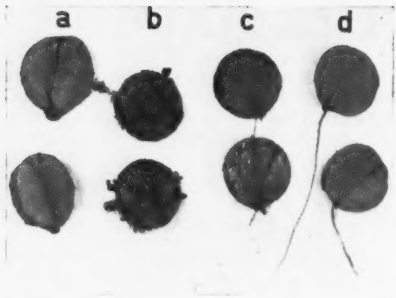



Fig. 1. Effect of (*a*) gibberellic acid ($10^{-6}$ gm./ml.), (*b*) kinetin ($5 \times 10^{-6}$ gm./ml.), (*c*) water (control), (*d*) 2,4-dichlorophenoxyacetic acid ($10^{-5}$ gm./ml.) on regeneration of leaf disks of *Begonia rex* six weeks after application. The disks (*c*) have the ventral surface uppermost.

or counteracted their promotion by 2,4-dichlorophenoxyacetic acid, but also abolished the polarity of shoot formation so that shoots were distributed over the whole area of both sides of the leaf. This latter effect was especially pronounced at a concentration of $5 \times 10^{-6}$ gm./ml. in the absence of added auxin (Fig. 1). Kinetin had no effect on the time of appearance of shoots.

A third type of hormone which could be expected to influence the regeneration process is gibberellic acid. It is known that it can react synergistically with auxins[2] and it has been shown that it can replace kinetin[3]. Addition of gibberellic acid in these experiments led to surprising results. In concentrations between $10^{-6}$ and $10^{-5}$ gm./ml. it inhibited both root and shoot formation. This inhibition could be relieved by addition of 2,4-dichlorophenoxyacetic acid at the same concentrations respectively; under these conditions the ability of the disks to form shoot and roots was roughly the same as in the controls. In contrast to 2,4-dichlorophenoxyacetic acid, kinetin was unable to counteract the inhibitory effect of gibberellin.

We thank Dr. Levens of the Lilly Research Laboratories, Indianapolis, for providing us with a sample of gibberellic acid.

H. Schraudolf
J. Reinert

Department of Botany,
University of Tübingen.

[1] Skoog, F., and Miller, C. O., *Symposia Soc. Exp. Biol.* (Cambridge) **11**, 118 (1957).
[2] Stowe, B., and Yamaki, T., "Ann. Rev. Plant Physiol.", **8**, 181 (1957).
[3] Vasil, J. K., *Science*, **126**, 1294 (1957).

## Fertilization of Rabbit Ova *in vitro*

In reviews of the evidence for mammalian fertilization *in vitro*, Austin and Bishop[1] stated that "it seems best for the present to regard the case as *sub judice*". Chang[2] concluded that "up till now we still do not have a repeatable procedure to fertilize mammalian eggs *in vitro*". Since the recognition of 'capacitation' of spermatozoa in the female tract by Chang[3] and Austin[4], Thibault and his associates[5–7] have reported cytological evidences of fertilization of rabbit ova *in vitro* by capacitated sperms. It was thought that unless living young are obtained by transplanting such fertilized ova into recipient rabbits, fertilization *in vitro*, as determined by cytological evidences, may not be sufficiently proved because such ova may be abnormally and/or incompletely fertilized, may die during the process, or may not be fertilized at all. This note reports a procedure to fertilize rabbit ova *in vitro* and the probability of normal development *in vivo* of such *in vitro* fertilized rabbit ova.

An œstrous rabbit was bred three times by fertile bucks at about 9.00 p.m. for the recovery of capacitated sperms, and two other rabbits were injected intravenously with sheep pituitary extract to induce ovulation for the recovery of unfertilized ova. Next day at about 9.00 a.m., before killing, an animal was bled by heart puncture or from the carotid artery in order to obtain fresh serum for the culture of ova. About 3–5 ml. of freshly prepared Krebs-Ringer bicarbonate solution containing 0·25 per cent of glucose was injected into one uterine horn of the mated rabbit and the fluid withdrawn immediately and placed into 1·5 ml. capacity small Carrel flasks. Progressive motile sperms from the uterine washings could be seen in most cases. The Fallopian tubes of the other two rabbits were flushed with Krebs-Ringer bicarbonate solution and the ova (still in mucous clot) were placed into a small Carrel flask that contained uterine sperms. These flasks were stopped with rubber and attached to a gentle rocking device placed inside an incubator at 38° C. After about 3–4 hr., the ova, free from the mucous clot but with corona cells still attached, were picked up with a capillary pipette and transferred into an 8 ml. capacity Carrel flask containing 4 ml. of 50 per cent heated rabbit serum (at 55° C. for 20 min.) in saline. After culture for another 18 hr. the ova were picked out, separated, mounted *in toto* on a slide[8] and examined under a compound microscope before fixation to determine the location of sperms. After fixation with acetic alcohol they were examined for the polar bodies, pronuclei, and the second maturation spindle, and then stained with Lacmoid for checking details.

The ova thus examined were classified into four groups: (*a*) Unfertilized; the ova that had definite second maturation spindles irrespective of the presence sperms. (*b*) Uncertain; those ova having no second maturation spindles but the nuclear configuration of which was at variance; some had one pronucleus, some had two groups of chromozomes, and some had several pronuclei. They may have been parthenogenetically activated, or fertilized, but died at an early stage. (*c*) Fertilized but dead; ova showing sperms on the zona or in the perivitelline space and with the nucleus at the anaphase of the second maturation division; some had a definite second polar body and had either cleaved into two cells or had two pronuclei, but most of them had fragmented. (*d*) Fertilized and cleaved normally; those cleaved into 4 cells and with either a second polar body or sperms in the perivitelline space.

Of 266 rabbit ova examined, 166 (62 per cent) were unfertilized, 23 (8·7 per cent) were uncertain, 22 (8·3 per cent) seemed to have been fertilized but died at an early stage, and 55 (21 per cent) cleaved normally and were considered definitely fertilized. Of these 51 ova, 36 were transplanted[9] into the tubes of 6 recipient rabbits that had been injected with pituitary extract 18 hr. previously. The recipient animals were allowed to deliver at term. Two recipients did not become pregnant; but 4 delivered 15 living healthy young. The probability of normal development of such *in vitro* fertilized ova is then about 42 per cent.

The procedure used in the present study is similar to that recommended by Thibault[10], and the proportion of ova fertilized is similarly low. The concentration of sperms in the uterine washings ranged from 10,000 to 26,000 per ml. and the proportion of fertilized ova was not correlated with the concentration of sperms. Furthermore, when uterine washings were centrifuged to concentrate sperms, or when a small amount of saline was used to wash the uterine horns

the proportion of ova fertilized was not increased. The proportion of fertilized ova was also not increased by using sperms recovered from the tubes of a mated animal (at 10,000 to 15,000 sperms per ml.). In this case only 5 out of 41 ova (12·2 per cent) were fertilized. Autologous serum seems to be better than heterologous serum for culturing newly fertilized ova: when autologous serum was used 50 out of 102 ova (49 per cent) were fertilized and 43 of the 102 (42 per cent) cleaved normally; but in heterologous serum 18 of 109 ova (17 per cent) were fertilized and 11 of the 109 (10 per cent) cleaved normally.

Due to the thick layer of corona cells on the zona pellucida, it was not possible to observe the penetration of sperm through the zona. Judging from their rate of cleavage, as compared with those ova recovered from the mated rabbit and cultured similarly, penetration of sperms probably occurred when the ova were in saline rather than in serum. In some experiments, the zona pellucida at the time of examination was very soft or partially dissolved. This may be due to a reaction between the zona and some factors in the uterine washing, as it was shown that the zona of unfertilized ova dissolved in a few hours when transplanted into the uterus[11] or it may be that certain factors in the serum of a particular animal affect the zona in this way. At the time of examination the general appearance of a fertilized ovum is better than that of an unfertilized ovum. This shows that fertilization increases the resistance of the ovum to the artificial medium.

Although the proportion of fertilized ova is relatively low and sometimes no fertilization occurred due to infection or other unknown reasons under the present experimental conditions, it can be said that at least we have a repeatable procedure for fertilizing mammalian ova *in vitro* and that such ova are truly fertilized and able to develop into normal young. Further studies are planned to elucidate the mechanisms of mammalian fertilization *in vitro*.

This work was supported by the Population Council and the Dickinson Research Memorial, Planned Parenthood Federation of America. Thanks are due to Miss Dorothy M. Hunt for assistance, and to Dr. G. Pincus for constant interest.

M. C. Chang

Worcester Foundation for Experimental Biology, Shrewsbury, Mass. and Department of Biology, Boston University, Boston, Mass.

[1] Austin, C. R., and Bishop, M. W. H., *Biol. Rev.*, **32**, 296 (1957).
[2] Chang, M. C., In "The Beginnings of Embryonic Development". Amer. Assoc. Adv. Sci. Symposium, Washington, D.C., 109 (1957).
[3] Chang, M. C., *Nature*, **168**, 697 (1951).
[4] Austin, C. R., *Aust. J. Sci. Res.*, B, **4**, 581 (1951).
[5] Dauzier, L., Thibault, C., and Winterberger, S., *C. R. Acad. Sci.*, **238**, 844 (1954).
[6] Thibault, C., Dauzier, L., and Winterberger, S., *C. R. Soc. Biol.*, **148**, 789 (1954).
[7] Dauzier, L., and Thibault, C., Third Int. Congr. Anim. Reprod., Cambridge, Sect. I, 58 (1956).
[8] Chang, M. C., *J. Exp. Zool.*, **128**, 379 (1955).
[9] Chang, M. C., *J. Exp. Zool.*, **144**, 197 (1950).
[10] Thibault, C. (personal communication, 1956).
[11] Chang, M. C., In "La Fonction Tubaire et ses Troubles" 140 (Masson et Cie, Paris, 1955).

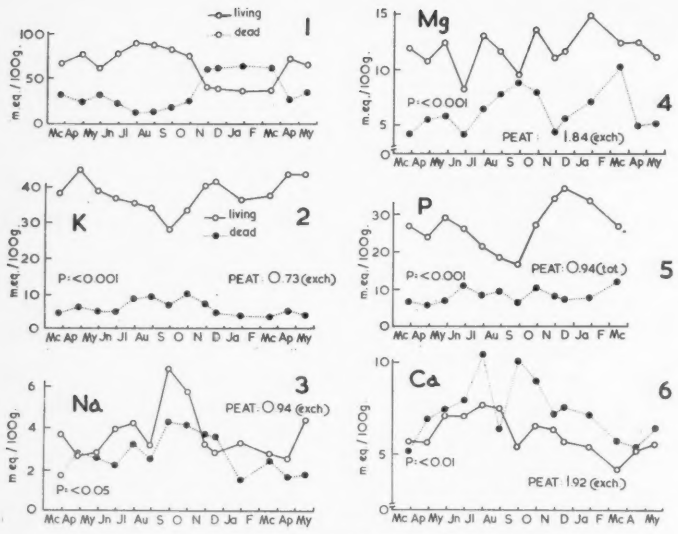
## Mineral Uptake and Retention in Cotton-grass (*Eriophorum vaginatum* L.)

Several investigations[1–4] have indicated the importance of mineral nutrients in determining the characteristic flora and vegetation structure of the various types of bog and fen. These and other extensive examinations of the nutrient levels in the underlying peat and water have not yet been followed, however, by a parallel study of the actual amounts of macro-elements taken up throughout the year by the principal mire species themselves, nor has their rate of turnover of mineral ions been investigated.

In view of the extremely low nutrient status of raised and blanket bogs, where the only source of salts is apparently via dust and sea-spray blown inland[4,5], nutrient supply, accumulation and turnover relationships may be at their most critical and, accordingly, we have chosen for the present study a typical bog species, *Eriophorum vaginatum* L. obtained from a site near Moel Llyfnant, Merionethshire, (altitude 1,400 ft.). The needle-like leaves of this species grow from the base and die back from the tip, eventually falling over to become incorporated in the litter layer. Duplicate monthly samples were collected by hand pulling, over a fourteen-month period 1954–5, dead leaves and dead leaf portions were separated from living leaf material, each being dried and ground prior to analysis. Although both growth and die-back occurred on a small scale simultaneously throughout the year in the *Eriophorum* community, Fig. 1 indicates that there was an overall seasonal pattern of autumnal die-back followed by a spring regrowth.

Sodium and potassium determinations were made by flame-photometry on diluted ash solutions; calcium was also measured in this way by a method developed in this laboratory to minimize interference. Magnesium and phosphorus were determined photometrically using titan-yellow and molybdate/stannous chloride methods, respectively. The glucose formed after one hour in boiling *N* sulphuric acid (R. E. Deriaz, Grass. Res. Inst. Hurley, private communication) was determined photometrically using an anthrone procedure, and a figure for 'carbohydrate' calculated. The mineral-levels are graphed as milliequivalents per 100 gm. of 'carbohydrate' free dry leaf material as shown, the 'carbohydrate' correction being an attempt to eliminate errors due to seasonal variations in photosynthetic activity, etc. The results of our mineral analyses are in general agreement with those of Thomas and Trinder[6] when allowance is made for the fact that they analysed unsorted leaf material collected throughout the growing season and expressed the results on a total dry matter basis.

Samples of peat, taken at rooting level, were analysed for total phosphorus and cations exchangeable with *N* ammonium acetate, and the results which are similar to those of Gore and Allen[7] included in the appropriate graphs as milliequivalents per 100 gm. dry peat. Values for the ratio of average nutrient in living or dead material to the nutrient value for peat are as follows: potassium (living material) 52, (dead) 8; phosphorus (living) 28, (dead) 9; sodium (living) 4, (dead) 3; magnesium (living) 6, (dead) 3; calcium (living) 3, (dead) 4. These ratios, considered together with the graphed results, indicate that *Eriophorum* concentrates potassium and phosphorus to a marked degree and further, irrespective of any of their seasonal fluctuations in living leaves, these elements are translocated away prior to die-back and thus not wholly incorporated into the litter. The ratios and graphed results for magnesium and sodium also suggest the possibility of a similar concentration and retention of these elements by living leaf tissue. The apparent increase in the calcium content of the dead material may be due to a fall in dry weight caused by breakdown of protein prior to die-back. The results for




Figs. 1–6. 1. Annual fluctuation in living and dead fractions of leaf material per 100 gm. dry matter. 2–6. Mineral nutrient content of living and dead leaf material throughout the year as milliequivalents per 100 gm. "Carbohydrate" free dry matter. Exchangeable (*exch.*) or total (*tot.*) nutrient content of peat included on appropriate graph as milliequivalents per 100 gm. dry peat (equivalent weight of phosphorus = 10·33). *P*, level of significance of difference between mean mineral content of dead and living material.

potassium and calcium are in line with those obtained for *Fagus sylvatica* L. by Olsen[8] who, however, observed no autumnal mobility of phosphorus or magnesium. Other experiments to be described elsewhere, suggest that the lower mineral values in the dead material were not caused by the 'washing out' effects of rainfall, a conclusion also reached for the beech[8].

Thus the phenomenon of mineral retention may possibly be an important factor in extending the range of nutrient conditions under which *E. vaginatum* can successfully grow. Tussocks or 'islands' of this species may be regarded as nutrient reservoirs particularly of phosphorus and potassium, an item of practical significance as both leaves and peduncles of this species are extensively grazed by certain breeds of hill sheep, especially during the 'hungry-gap' period of early Spring. Mineral accumulation may also be a contributory factor to the successful way in which *Calluna* and other species colonize moribund *E. vaginatum* tussocks. In view of the strong potassium retention by living leaf tissue it would be interesting, in relation to radioactive fall-out, to investigate whether cæsium behaves in a similar manner.

We are indebted to the Agricultural Research Council for a grant to assist this work, to the Royal Society for the provision of a flame-photometer and to Mr. L. Pugh for assistance in the collection of material.

Gordon T. Goodman
Donald F. Perkins

Botany Department
University College of Swansea.
June 3.

[1] Gorham, E., *Oikos*, **2**, 217 (1950).
[2] Sjörs, H., *Oikos*, **2**, 241 (1950).
[3] Gorham, E., and Pearsall, W. H., *J. Ecol.*, **44**, 129 (1956).
[4] Gorham, E., *J. Ecol.*, **44**, 142 (1956).
[5] Gorham, E., *Geochim. et Cosmochim. Acta*, **7**, 231 (1955).
[6] Thomas, B., and Trinder, N., *Emp. J. Exp. Agric.*, **15**, 237 (1947).
[7] Gore, A. J. P., and Allen, S. E., *Oikos*, **7**, 48 (1956).
[8] Olsen, C., *Compt. Rend. Lab. Carlsberg, Sér. Chim.*, **26**, 197 (1948).

## Prevention of the Onset of Seed Dormancy by Gibberellic Acid

One of the striking properties of gibberellic acid is its ability to curtail the rest period of seeds and other dormant organs. For example, dormant seeds of lettuce[1], peach[2], *Arabidopsis*[3] and barley[4] germinate spontaneously when placed in solutions of gibberellic acid. However, no data are available concerning the effects of this substance on the onset of seed dormancy when plants bearing developing seeds are treated, although such effects might be predicted. Results obtained in this laboratory bear out this prediction.

For this study an inbred strain of *Avena fatua* was chosen. Seeds of this strain exhibit very deep dormancy such that neither the removal of the hull nor an incision into the seed (in air) has any beneficial effect in promoting germination. Further, even isolated embryos germinate only when supplied with gibberellic acid, or after prolonged leaching, or in an oxygen-enriched atmosphere. Thus, these seeds possess true embryo dormancy.

For these experiments plants were grown in a greenhouse during March–June, 1959. When the endosperm of the seeds had reached the 'milk stage' the stems were cut below the node of the youngest leaf and the cut end placed in an Erlenmeyer flask containing 200 ml. of an aqueous solution of gibberellic acid (potassium salt). In the preliminary experiment three plants were placed in each flask containing the concentrations 0, $10^{-1}$, 1·0, 10, 100, or 1000 p.p.m. gibberellic acid and kept in the greenhouse. When the solutions had been taken up, the flasks were replenished with distilled water. The seeds were collected at maturity (8–10 days after initiation of the experiment) and after a further 2 days they were tested for germination capacity. In this test seeds were placed in Petri dishes on filter paper moistened with a standard volume of a mixture of antibiotic solutions (Candidin 250 p.p.m., Neomycin 20 p.p.m.) which prevented fungal and bacterial growth, and kept in darkness at 20° C. and 100 per cent relative humidity. Twenty intact 'seeds' (caryopsis surrounded by hull) and 20 isolated caryopses were tested in each case. After 10 days the percentage germination of caryopses from plants treated with 10, 100 and 1,000 p.p.m. were 75, 100 and 100 per cent respectively. Similarly, intact 'seeds' germinated 30, 25 and 50 per cent respectively. The intact 'seeds' and isolated caryopses from those plants treated with distilled water did not germinate even after 20 days in the germination test.

This experiment was repeated using the same technique except that 6 plants were placed in each flask containing either 200 ml. distilled water, 10, 100, or 1000 p.p.m. gibberellic acid. After harvesting, the germination capacity of isolated caryopses was tested. For each experimental group, two replicates both consisting of 50 caryopses were employed.

The results shown in Fig. 1 indicate the rate of germination. Clearly, treatment of plants with gibberellic acid resulted in the production of non-dormant seeds. The effect here is particularly remarkable when it is remembered that even isolated embryos of seeds from normal, untreated plants are strongly dormant.

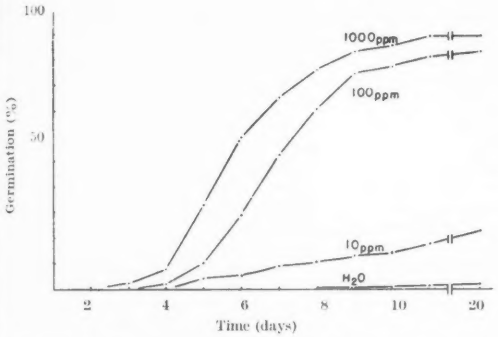



Fig. 1. Germination of isolated caryopses from plants treated with water, 10, 100, or 1000 p.p.m. gibberellic acid. Points on the curves represent the mean of 2 replicates each comprising 50 embryos. The standard deviation for all points was less than 2

The most plausible and simplest explanation of these results is that gibberellic acid was transported into the seeds during development, thus rendering them capable of spontaneous germination when placed subsequently under germination conditions.

In these experiments the gibberellin content of the seeds was augmented artificially. We have found that seeds of this species contain gibberellin-like substances, and presumably, if in the development of the seeds the level of these naturally occurring gibberellins were increased there would likely be no rest period. Further, it might be pointed out that results of experimental work in progress in this laboratory indicate that the beneficial effect of gibberellic acid depends on an antagonism between this substance and an endogenous inhibitor. It is an interesting speculation that differences in the level of naturally occurring gibberellins and inhibitors might be of general significance in the onset and maintenance of seed dormancy.

A similar effect has been reported by Lippert and his co-workers[5] who produced non-dormant potato tubers by treating the tuber-bearing plants with gibberellic acid solution.

This work was supported by an extra-mural research grant from the Canadian Department of Agriculture.

M. Black
J. M. Naylor

Department of Field Husbandry,
University of Saskatchewan,
Saskatoon, Canada.
July 14.

[1] Kahn, A., Goss, J. A., and Smith, D. E., *Science*, **125**, 645 (1957).
[2] Donoho, C. W., and Walker, D. R., *Science*, **126**, 1178 (1957).
[3] Kribben, F. J., *Naturwiss.*, **44**, 313 (1957).
[4] Pollock, J. R. A., *Chem. and Ind.*, 387 (1958).
[5] Lippert, L. F., Rappaport, L., and Timm, H., *Plant Physiol.*, **33**, 132 (1958).

## MICROBIOLOGY

### Microbiological Transformation of a Cardiac Aglycone

It has been shown recently by Brown and his co-workers[1] that when digitoxin was administered to rats or to adult humans some of it underwent hydroxylation at $C_{12}$ to give digoxin. This fact, suggestive of the possibility of such bioconversions to be effected by micro-organisms as well, prompted us to investigate microbiological transformation of the cardiac glycosides and their aglycones. In this communication we wish to report that the $C_{12}$- and $C_{16}$-hydroxylations of digitoxigenin.

Incubation of digitoxigenin was conducted with cultures of various species of micro-organisms. Extraction of the culture broths with ethyl acetate, followed by concentration of the solution afforded crude extracts which were submitted to the examination of products by paper partition chromatography under the following conditions: Toyo Filter Paper No. 51; developing solvents, system 1, benzene: ethyl acetate: water (8:5:4)[2], system 2, benzene:methanol: water (5:3:2)[2]; ascending method at 20° C.; reagents, trichloroacetic acid[3] and 3,5-dinitrobenzoic acid[4]. Each case was accompanied by blank incubation (that is, incubation with no substrate) for the purpose of comparison. The results of the experiments showed that at least three fungi among the strains of micro-organisms examined, namely *Helicostylum piriforme* Bainier, *Cunninghamella blakesleeana* Lendner, and *Gibberella fujikuroi* (Saw.) Wr., were able to convert digitoxigenin into other substances.

*Helicostylum piriforme:* On the paper chromatogram were observed two distinct spots, one of which had an $R_F$ of 0·70 (system 1) or 0·12 (system 2) and the other an $R_F$ of 0·49 (system 1) or 0·05 (system 2). These $R_F$ values were indistinguishable from the respective $R_F$ values obtained on concurrent chromatography of authentic gitoxigenin and digoxigenin. The rate of formation of digoxigenin seemed fairly higher than that of gitoxigenin. Under other fermentation conditions, these products were not detected but another spot was found having an $R_F$ of 0·35 (sytem 2), formation of which was more evident in the case of *Cunninghamella*.

*Cunninghamella blakesleeana:* Two intense spots of products appeared on the paper chromatogram. One of them exhibited an $R_F$ of 0·80 (system 1) or 0·35 (system 2) which was not consistent with any of the $R_F$ values of the so-far-known aglucones of digitalis glycosides and the product awaits further scrutiny. The other of the two spots showed an $R_F$ of 0·68 (system 1) or 0·12 (system 2) and coincided in $R_F$ value with authentic gitoxigenin. The yield of the latter product was apparently somewhat lower than in the former case.

*Gibberella fujikuroi:* The product had an $R_F$ of 0·46 (system 1) or 0·03 (system 2) and was identical with authentic digoxigenin.

Gitoxigenin and digoxigenin thus identified were further confirmed by comparison of their coloration and fluorescence on paper chromatogram and other properties with those of respective authentic samples.

Thus, we were able to find three strains of micro-organisms which convert digitoxigenin into gitoxigenin, digoxigenin, or an unknown product, and could open up future possibilities of microbiological transformation of cardiac glycosides for obtaining more useful compounds.

While this work was in progress, Gubler and Tamm[5] reported the $C_{12}$-hydroxylation of digitoxigenin and gitoxigenin with *Fusarium lini*. Our repetition of their experiments using *F. lili* Bolley led to identical results; and through a personal communication from Prof. M. Ishidate of the University of Tokyo, we have learned recently that his research group has found independently the $C_{12}$-hydroxylation of digitoxigenin effected by a species of *Gibberella* in good yield.

This work was undertaken with the assistance of Messrs. A. Okabori, K. Kishino, and T. Takahashi.

Hayao Nawa
Masao Uchibayashi
Takaaki Kamiya
Research Laboratories,
Takeda Pharmaceutical Industries, Ltd.
Togo Yamano
Hideo Arai
Matazo Abe
Institute for Fermentation, Osaka,
Osaka, Japan.
June 8.

[1] Brown, B. T., Wright, S. E., and Okita, G. T., *Nature*, **180**, 607 (1957). Later K. Repke also obtained the same results, *Naturwiss.*, **45**, 94, 366 (1958).
[2] Sasakawa, Y., *Yakugaku Zasshi*, **75**, 946 (1955).
[3] Jensen, K. B., and Tønnöe, K., *J. Pharm. Pharmacol.*, **7**, 334 (1955).
[4] Tschesche, R., Grimmer, G., and Seehofer, F., *Chem. Ber.*, **86**, 1235 (1953).
[5] Gubler, A., and Tamm, C., *Helv. Chim. Acta*, **41**, 297, 1762 (1958).

## Survival of Spermatozoa Following Drying

Just ten years ago, *Nature* published a report of the survival of spermatozoa following freezing in the presence of glycerol[1], a discovery of major importance which afterwards led to the preservation by freezing of a wide range of mammalian tissues. This procedure has proved eminently successful for long-term storage, its only drawbacks being the necessity for the introduction of glycerol and the need for very low temperatures of storage. For these reasons, the preservation of non-glycerolated living cells in the dried state at room temperature has added virtue in terms of practicability. This communication reports the successful accomplishment of the first step toward such an end; namely, the recovery of a high percentage of living cells following freeizng, drying, and reconstitution without storage.

Previously reported attempts to freeze-dry spermatozoa[1,2] have started with glycerolated material on the assumption that spermatozoa cannot otherwise survive the initial freezing. Unfortunately the desiccation of such material leaves the cells suspended in concentrated glycerol in which storage at room temperature is not presently feasible. However, as the subsequent experiment indicates, there do appear to be conditions under which good survival of motile spermatozoa can be expected following freezing in the absence of glycerol, making the preparation of truly dried material possible.

Freshly collected bull semen is diluted approximately 20 to 1 with heated, filtered whole milk or with a citrate egg yolk extender containing 2.9 gm. sodium citrate and 22 ml. egg yolk made up to 100 ml. with distilled water. The diluted semen is taken up on a sheet of nylon gauze roughly 1 × 3in. in dimension with a 1 mm. mesh. This gauze is hung in a glass vessel 4 in. long and 2 in. in diameter which is connected through a stopcock with an 8-mm. bore to the inlet of a mechanical vacuum pump with a free-air pumping speed of 27 cu. ft./min. With the vacuum pump running the stopcock is abruptly opened. The temperature of the semen preparation falls from 25° C. to between −10° and −15° C. within 3 sec., freezes with an abrupt rise in temperature to −5° to −6° C. and then falls within 5 sec. to −30° C. Drying is completed in 5–6 min. During this time the temperature of the specimen is rising slowly, reaching −25° three minutes following freezing, then rising more rapidly to approach room temperature. On removal from the vacuum chamber the material is white and powdery. It is immediately reconstituted in an excess of the original extending medium. Because of the dilution resulting it is necessary to centrifuge the reconstituted material in order to concentrate the spermatozoa for assay of the results.

Fresh bull semen processed as described yields a product in which 40–50 per cent of the spermatozoa are motile. The recovery of semen with low initial motility or of that processed more than 8 hr. after collection is substantially reduced. A single insemination has been carried out with material frozen, dried, and reconstituted as described above. This sample contained roughly 45 per cent motile sperm with 30 per cent progressive motility. The insemination was made with 1 ml. of suspension containing approximately one million cells. This experiment has resulted in fertilization.

The detail with which the procedure is described above is necessary because of the narrow limits within which this essentially empirical process is successful. It may perhaps be some consolation to the many investigators who have unsuccessfully attempted this procedure in the past that we must admit to an extraordinary degree of good fortune in the selection of experimental conditions. The identical procedure carried out with a pump of half the capacity yielded only occasional motile sperm. With a pump of more than five times the capacity, results were wholly negative. Subsequent preliminary experiments indicate the necessity for discriminating between freezing and drying. Results are improved if the rate of freezing is reduced by constricting the pumping orifice until freezing has taken place. However, a similar reduction in pumping speed during the drying results in a total loss of motile sperm.

It is well known from many past experiences that excessive rates of freezing are destructive to spermatozoa so that it is not surprising to find this still true for freezing by sublimation. Whether the successful freezing of non-glycerolated material is due simply to a fortuitous choice of freezing rate or whether the simultaneous dehydration plays a protective part needs further study. The latter probability is suggested by past studies on partially dehydrated cells[3] and by our concurrent studies with erythrocytes. On theoretical grounds one might also have predicted that rapid drying would be desirable. The interval between freezing and the completion of drying is, after all, a period of storage in the frozen state for undried portions and −30° C. is too high a temperature to be fully stabilizing.

Only a few isolated storage experiments have been conducted. Material dried as described and stored for periods of 1, 3, and 7 days at room temperature in a desiccator containing silica gel showed occasional motile sperm on reconstruction after 24 hr. storage with no further loss in the 3- and 7-day storage experiments. This justifies some degree of optimism regarding room-temperature storage, and we anti-

cipate that a study of preservation under controlled conditions of water vapour pressure should enable a substantial improvement in recovery following storage.

We wish to express our appreciation for the assistance of Mr. H. F. Harner and Mr. W. L. Campbell of the Maryland-West Virginia Artificial Breeders Co-operative in supplying experimental material and in carrying out the insemination.

The opinions or assertion contained herein are our own, and are not to be construed as official or reflecting the views of the Navy Department or the Naval Service in general.

HAROLD T. MERYMAN
EMANUEL KAFIG

Naval Medical Research Institute,
National Naval Medical Center,
Maryland,
Bethesda

[1] Polge, C., Smith, A. V., and Parkes, A. S. *Nature*, **164**, 666 (1949).
[2] Albright, J. L., Erb, R. E., and Ehlers, M. H., *J. Dairy Sci.*, **41**, 206 (1958).
[3] Luyet, B. J. and Hodepp, E. L. *Proc. Soc. Exp. Biol., N.Y.*, **39**, 433 (1938).

## Fractionation of the System Bringing About Oxidative Phosphorylation in *Azotobacter vinelandii*

RESPIRATORY-CHAIN phosphorylation in a particulate fraction of *Azotobacter vinelandii* is inactivated by incubation of the suspension in salt concentrations less than 0·01 *M* potassium chloride or sodium chloride, or 0·0008 *M* magnesium chloride, manganese chloride or calcium chloride. This inactivation is partially reversed by adding salts back to the inactivated suspension[1]. It has now been found that the inactivated suspension can be fractionated by centrifugation at 50,000 *g* for 30 min. The sediment contained 85–90 per cent of the reduced diphosphopyridine nucleotide oxidase activity, but restoration of oxidative phosphorylation was not possible unless the suspension was pre-incubated with the supernatant from this high-speed centrifugation, as well as with magnesium chloride (Table 1). Although after pre-incubation this supernatant alone also catalysed oxidative phosphorylation, the oxidase activity was much too low to account for the increased P:O ratio obtained with particles pre-incubated with magnesium chloride and supernatant. It appears, therefore, that the supernatant contains a factor which is necessary for the restoration of the activity of inactivated particles. This factor is destroyed by heating for 5 min. at 100°.

Table 1. REACTIVATION OF OXIDATIVE PHOSPHORYLATION BY A SOLUBLE COMPONENT

| Experiment | Pre-incubation mixture: WSP | WSP′ | WSP″ | $MgCl_2$ (0·008 *M*) | Serum albumin (0·1%) | $S_2$ | Oxidative phosphorylation: Time needed to complete oxidation (minutes) | P:O ratio |
|---|---|---|---|---|---|---|---|---|
| 1 | + | — | — | + | + | — | <3 | 0·51 |
| | — | + | — | + | + | — | <3 | 0·02 |
| | — | + | — | + | + | — | <2·5 | 0·36 |
| | — | — | + | + | + | — | 3 | 0·03 |
| | — | — | + | + | + | + | 2·5 | 0·19 |
| 2 | + | — | — | + | + | — | 5·5 | 0·67 |
| | — | + | — | + | + | — | <3·5 | 0·16 |
| | — | + | — | + | — | — | <2·5 | 0·62 |
| | — | — | + | + | + | — | 2·5 | 0·08 |
| | — | — | + | + | + | + | 2 | 0·26 |
| | — | — | + | + | + | +* | 2·5 | 0·11 |
| | — | — | + | + | + | ++ | <3 | 0·10 |
| | — | — | + | + | + | +‡ | 13 | 0·19 |
| | — | — | — | + | + | +‡ | 13 | 0·40 |

WSP, washed small particles obtained by centrifugation for 2 hr., at 140,000 *g* (bottom of tube) (ref. 9); suspended in 0·05 *M* Sørensen phosphate buffer, *p*H 7·0. WSP′, washed small particles suspended in 0·005 *M* phosphate buffer, *p*H 7·0, for 30–90 min., at 0°. WSP″, sediment after centrifugation of WSP′ for 30 min. at 50,000 *g*. $S_2$, supernatant obtained from this centrifugation.

The mixtures indicated were pre-incubated for 90–120 min. at 0°, and were then added to a reaction medium[9] used to measure oxidative phosphorylation with reduced diphosphopyridine nucleotide as substrate.

* $S_2$ was heated at 100° for 5 min. and filtered.

† $S_2$ was not present in the pre-incubation mixture, but was added immediately before the measurement of oxidative phosphorylation.

‡ Three times as much $S_2$ as used in the measurements with WSP″.

This fractionation resembles that carried out by Pinchot[2] with extracts of *Alcaligenes faecalis*. The conditions leading to the reversible inactivation of the phosphorylating system in *Azotobacter* are similar to those which bring about a reversible dissociation of a two-stranded polynucleotide complex[3], or, so far as decreasing the magnesium concentration is concerned, to the dissociation of ribonucleoproteins in particles obtained from yeast[4] and *Escherichia coli*[5]. This provides some support for Pinchot's[6] suggestion that in his preparations a polynucleotide acts as a bridge holding together the necessary enzymes. It is possible that a factor necessary for oxidative phosphorylation in the particles obtained from *Azotobacter* is bound to the particles by means of such a polynucleotide complex, which dissociates on lowering the cation concentration.

These experiments with *Azotobacter* recall also recent reports on the fractionation of the phosphorylating enzymes in particles derived from beef-heart mitochondria[7,8]. Phosphorylation was obtained by bringing together a particulate fraction (containing the oxidase), a soluble fraction and magnesium. Linnane[8] found that no fractionation took place in the presence of magnesium.

H. G. HOVENKAMP

Laboratory of Physiological Chemistry,
University of Amsterdam.
July 24.

[1] Hovenkamp, H. G., *Biochim. Biophys. Acta*, **34**, 485 (1959).
[2] Pinchot, G. B., *J. Biol. Chem.*, **205**, 65 (1953).
[3] Felsenfeld, G., and Rich, A., *Biochim. Biophys. Acta*, **26**, 457 (1957).
[4] Chao, F. H., *Arch. Biochem. Biophys.*, **70**, 426 (1957).
[5] Tissières, A., and Watson, J. D., *Nature*, **182**, 778 (1958).
[6] Pinchot, G. B., *J. Biol. Chem.*, **229**, 25 (1957).
[7] Pullman, M. E., Penefsky, H., and Racker, E., *Arch. Biochem. Biophys.*, **76**, 227 (1958).
[8] Linnane, A. W., *Biochim. Biophys. Acta*, **30**, 221 (1958).
[9] Tissières, A., Hovenkamp, H. G., and Slater, E. C., *Biochim. Biophys. Acta*, **25**, 336 (1957).

## Transformation Reaction of Pneumococci in the Absence of Serum Factor

CONTRARY to the general proposition that transformation reactions of pneumococci cannot take place in the absence of serum factor[1–3], our experiments[4,5] indicated that cells of *R36NC*, a pneumococcal rough strain derived from 11-*D39S*, could be transformed to streptomycin-resistant ones by means of purified deoxyribonucleate in diffusate media. But in these experiments, deoxyribonucleate was kept present throughout the culture growth and the cultivation was continued overnight before plating on streptomycin plates, so that the population change after the occurrence of transformation reaction might have distorted the results. This possibility was completely excluded in the present experiment by the use of deoxyribonuclease, which was added to the reaction mixture to stop the action of deoxyribonucleate at a definite time.

Streptomycin-sensitive *R36NC* from a blood agar slant was inoculated into Adams and Roe's medium[6] and incubated overnight. The culture was added next morning to 4 volumes of fresh medium and

incubated for about 2 hours more until it showed good visible growth. One part of the preliminary culture and 17 parts of the medium were mixed and 1·8 ml. of the mixture was distributed in test-tubes and incubated at 37° C. At a definite time 0·2 ml. of deoxyribonucleate solution (100 μgm. per ml.) was added and 30 minutes later its action was stopped by adding one drop of deoxyribonuclease solution. The cultures were incubated further for 90 minutes in order to allow the streptomycin resistance to develop completely. One of the results is shown in Table 1. The

Table 1. Time of Appearance of Competent Cells.

| Time of exposure to deoxyribonucleate (hr min.)* | Number of resistant colonies (Quadruple experiments)† | | | |
|---|---|---|---|---|
| 0·00–0·30 | 0 | 0 | 0 | 0 |
| 0·30–1·00 | 0 | 0 | 0 | 0 |
| 1·00–1·30 | 0 | 0 | 0 | 0 |
| 1·30–2·00 | 0 | 0 | 0 | 0 |
| 2·00–2·30 | 18 | 0 | 0 | 0 |
| 2·30–3·00 | ‡ | ‡ | ‡ | 25 |
| 3·00–3·30 | ‡ | ‡ | § | 204 |
| 3·30–4·00 | § | 129 | 13 | 0 |
| 4·00–4·30 | 0 | 0 | 0 | 0 |
| 4·30–5·00 | 0 | 0 | 0 | 0 |

* Time from the start of the cultures.
† Number of resistant colonies which appeared from 0·1 ml. of the culture containing no serum.
‡ Colonies covering larger part of plate.
§ Isolated but innumerable colonies.

mean rate of transformation in 4 tubes between times 3·00 and 3·30, was about 0·05 per cent.

The effect of serum was next studied. The preliminary culture was grown in the absence of serum. In one series, one part of this culture, and 2 parts of normal horse serum (heated at 60° C. for 30 minutes) and 15 parts of the medium were mixed, and 1·8 ml. of this mixture was distributed in test-tubes. In another series, no serum was added. As shown in Table 1, the test-tubes under the same conditions contained varying numbers of transformants, so in this case 3 tubes were subjected to the same conditions. Immediately after the addition of deoxyribonuclease a definite volume of culture was taken from each tube and mixed, and all the viable cells in it were counted. The number of transformants in the mixture from

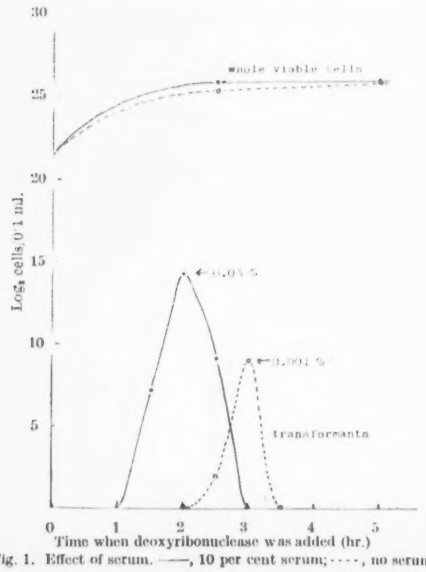



Fig. 1. Effect of serum. ——, 10 per cent serum; - - - -, no serum

3 tubes was counted after 90 minutes' incubation for phenotypic lag. Fig. 1 shows an example of these experiments. The number of whole viable cells in test-tubes containing serum was larger after 150 minutes' incubation than in those containing no serum. But more remarkable effects of serum were found in the number of transformants and their appearance. The competent cells, which are able to react with deoxyribonucleate, appeared about one hour earlier, were present for a longer period of time and were more numerous in the presence of serum than in its absence.

These results throw doubt on the claim that serum factor is essential for pneumococcal transformation. In the system used here serum, as in the case of *H. influenzae*[7], is not indispensable, but it may be an accessory factor, the effects of which appear to be related to the pattern of appearance of competent cells.

According to the generally accepted view, in order to study the mechanism of the transformation reaction in pneumococci, one has to consider 4 components; a competent strain, a transforming principle (deoxyribonucleate), a suitable medium for growth and serum factor. But the results of the present experiment have shown that the serum factor can be eliminated, and as a consequence the reaction system to be analysed has been made simpler.

Takeshi Odaka
Tadashi Watanabe

Institute for Infectious Diseases,
University of Tokyo.
May 19.

[1] McCarty, M., Taylor, H. E., and Avery, O. T., Cold Spring Harbor Symp. Quant. Biol., **11**, 177 (1946).
[2] Hotchkiss, R. D., and Ephrussi-Taylor, H., *Fed. Proc.*, **10**, 200 (1951).
[3] Fox, M. S., and Hotchkiss, R. D., *Nature*, **179**, 1322 (1957).
[4] Odaka, T., *Japan. J. Bact.*, **14**, 254 (1959) (in Japanese).
[5] Odaka, T., and Watanabe, T., *Japan. J. Exp. Med.* (in the press).
[6] Adams, M. H., and Roe, A. S., *J. Bact.*, **49**, 401 (1945).
[7] Alexander, H. E., and Leidy, G., *J. Exp. Med.*, **93**, 345 (1951).

## Incorporation of 5-Bromouracil into Transforming Principle of *Bacillus subtilis* and its Biological Effects

The incorporation of 5-bromouracil into deoxyribonucleic acids of bacteria and bacteriophage[1] raised a problem concernnig the biological effects of such incorporation. It has been shown that at least some of the cells of *Escherichia coli* containing 5-bromouracil in their deoxyribonucleic acid remain alive[2]. Further evidence on this subject was obtained by the demonstration of the mutagenic effect of 5-bromouracil[3]; these results also indicated that 5-bromouracil was incorporated into molecules of deoxyribonucleic acid that functioned as heredity determinants. Similar results have been obtained with bacteriophage.[4]

It appeared of interest to make a further study of the above problems by the incorporation of 5-bromouracil into deoxyribonucleic acid that has transforming activity. The incorporation of 5-bromouracil into deoxyribonucleic acid of *Hemophilus influenzae* thus far could not be demonstrated[5] presumably because this organism could not be grown under conditions in which it would require exogenous thymine. New possibilities have been opened by Dr. J. Spizizen's discovery of the transformation in *Bacillus subtilis*[6], an organism easily cultivated on synthetic media. In preliminary experiments, the cultivation of the wild strain of *B. subtilis* in enriched broth[7] containing 4 mgm. of 5-bromouracil per ml. did not result in a

demonstrable incorporation of this analogue into deoxyribonucleic acid. Use was therefore made of the fact that the uracil-requiring strain may also partially require thymine, and that aminopterin can be used to prevent endogenous methylation, thus resulting in an uptake of exogenous thymine analogue.[8]

As donor of deoxyribonucleic acid (transforming principle) served a uracil-requiring strain 265 of *B. subtilis*, kindly supplied by Dr. Robert Guthrie, Children's Hospital, Buffalo. The strain was grown with aeration at 37° for 20 hr. in enriched broth[7] containing 200 μgm. of 5-bromouracil and 200 μgm. of aminopterin per ml. The cells were washed five times with 0·85 per cent sodium chloride solution and resuspended in a similar solution made 0·05 *M* with respect to sodium citrate and containing 1 mgm of crystalline egg lysozyme per ml. The cell suspension was shaken 15 min. at 37°. It was observed that cells grown in the presence of 5-bromouracil and aminopterin were not lysed by lysozyme alone but did lyse upon the addition of a 15 per cent 'Duponol *C*' solution to obtain final 'Duponol' concentration of 5 per cent. This phenomenon is being investigated further.

Highly polymerized deoxyribonucleic acid was isolated and 5-bromouracil content therein determined as described in ref. 7. The molar ratio 5-bromouracil:5-bromouracil + thymine was 12 per cent (average of 4 determinations).

For preparation of the transforming principle an aliquot of isolated deoxyribonucleic acid was deproteinized, precipitated and stored as described in ref. 6.

The transforming principle prepared as above as well as the control one prepared in a similar way but with the omission of 5-bromouracil and aminopterin from the broth were used in transformation experiments performed as described in ref. 6, using as receptors the following strains: mutant 168 (indole⁻), kindly supplied by Dr. J. Spizizen; a methionine⁻ mutant, anarginine⁻ mutant and a pyridoxine⁻ mutant isolated in this laboratory after ultra-violet irradiation.

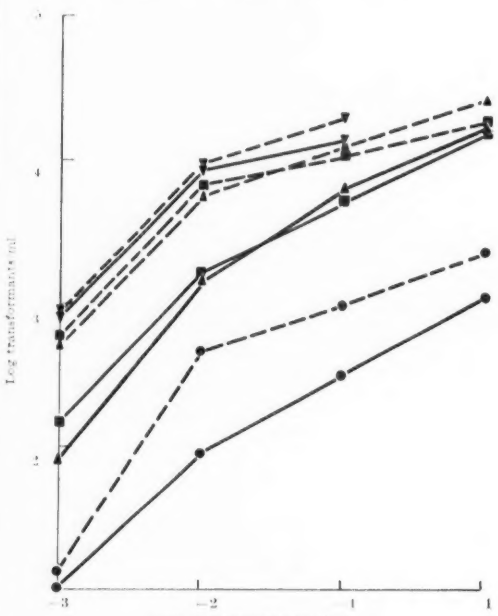



Fig. 1. Comparison of transforming activities between deoxyribonucleic acid containing 5-bromouracil and normal acid of *B. subtilis*. Dotted line, normal deoxyribonucleic acid; full line, acid containing 5-bromouracil. Transformation from dependence (−) to independence(+) for the following markers: arginine (●), pyridoxine (▲), methionine (■) and indole (▼).

Table 1. Transforming Activity (*Bacillus subtilis*) of Deoxyribonucleic Acid containing 12 Mole per cent of 5-Bromouracil

| DNA μgm./ml. | Transforming activity* | | | |
|---|---|---|---|---|
| | Pyridoxine | Arginine | Methionine | Indole |
| 1 | 42 (±10) | 28 (±10) | 93 (±4) | |
| 0·1 | 38 (±8) | 20 (±7) | 56 (±6) | 69 (±6) |
| 0·01 | 37 (±7) | 39 (±5) | 2+(±5) | 91 (±4) |

* In per cent of control without 5-bromouracil.

The results are represented in Table 1 (average of 4 transformation experiments) and in Fig. 1 (a typical experiment). First, it will be seen that, in general, the deoxyribonucleic acid containing 5-bromouracil has transforming activity. In the case of transformation from indole dependence to independence (indole 'marker'), the transforming activity is not significantly affected; in the cases of other 'markers', the activity is decreased, to a degree different for each 'marker'. These results could be interpreted to signify that either the activity of each 'marker' is affected differently[9] by the same amount of analogue or else that the amount of the analogue in each marker is different, for example if normally the amount of thymine in each marker is different.

We wish to thank Dr. J. Spizizen for his advice on the transformation of *B. subtilis*, and Mr. Kenneth Rich for technical assistance.

This work was supported by research grants from the National Institutes of Health, American Cancer Society and National Science Foundation.

Erela Ephrati-Elizur
Stephen Zamenhof

Department of Biochemistry,
College of Physicians and Surgeons,
Columbia University,
New York 32.
July 13

[1] Zamenhof, S., and Griboff, G., *Nature*, **174**, 306, 307 (1954). Dunn, D. B., and Smith, J. D., *Nature*, **174**, 305 (1954).
[2] Zamenhof, S., De Giovanni, R., and Rich, K., *J. Bacteriol.*, **71**, 60 (1956).
[3] Zamenhof, S., De Giovanni, R., and Greer, S., *Nature*, **181**, 827 (1958).
[4] Litman, R., and Pardee, A. B., *Nature*, **178**, 529 (1956).
[5] Greer, S. (in preparation).
[6] Spizizen, J., *Proc. U.S. Nat. Acad. Sci.*, **44**, 1072 (1958).
[7] Zamenhof, S., Reiner, R., De Giovanni, R., and Rich, K., *J. Biol. Chem.*, **219**, 165 (1956).
[8] Zamenhof, S., Rich, K., and De Giovanni, R., *J. Biol. Chem.*, **232**, 651 (1958).
[9] Zamenhof, D., Leidy, G., Greer, S., and Hahn, E., *J. Bacteriol.*, **74**, 191 (1957).

## Psittacosis Elementary Bodies

In the course of an investigation of the developmental cycle of psittacosis virus certain morphological features of the mature elementary bodies have been observed.

Changes occurring in the appearance of the virus during a single stage infective process were studied by electron microscopy of ultra-thin sections of chorioallantoic membrane of 8–10 day old chick embryos. The virus strain *M.O.H.* 154, originally obtained from Sir Sam Bedson, was adapted by repeated passage to grow on the chorioallantoic membrane, which in 24–48 hr. became œdematous and developed opaque white lesions. Membranes were harvested at timed intervals, fixed in a buffered osmium tetroxide solution and embedded in methyl and butyl methacrylate.

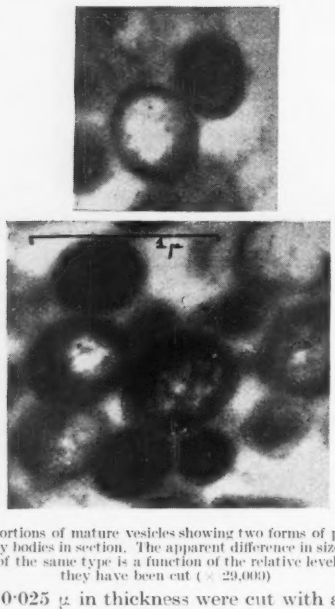


Fig. 1. Portions of mature vesicles showing two forms of psittacosis elementary bodies in section. The apparent difference in size between particles of the same type is a function of the relative level at which they have been cut (× 29,000)

Sections 0·025 μ in thickness were cut with a Porter-Blum[1] microtome and examined without further treatment in a Metropolitan-Vickers *E.M.* 6 electron microscope.

The final stage of psittacosis virus replication is the formation of one, or rarely two, vesicular inclusions packed with elementary bodies, which fill the host cell to the entire exclusion of cytoplasm. Rupture of the cell releases a large number of new infective particles into the intercellular spaces. A striking feature of the elementary bodies, both within the vesicles and lying free, as observed by high magnifications of the electron microscope, is that they exist in two distinct forms.

A thin section of a vesicle shows many elementary bodies and since the thickness of the section is approximately one tenth that of the diameter of an elementary body the latter are found to be cut at various levels. From a study of some hundreds of these sections there emerged a picture of the two forms. They are both spherical though of different size, the smaller having an average diameter of 0·25–0·3 μ, the larger 0·4–0·5 μ. The smaller is a relatively electron-dense structure with an outer membrane, which in some cases appears to be double, beneath which lies a thick layer of dense material. Inside this layer is a clearer zone, and in the centre of the body a dense spherical mass occupies one third to one half of the diameter of the whole. In the other form a limiting membrane immediately surrounds a layer of granular material, which, in turn encloses and merges with a relatively large central diffuse granular zone. The latter becomes progressively less dense towards the centre, and not infrequently appears to be organised into an irregular reticulum, some strands of which are sufficiently well defined to resemble septa.

The relative numbers of the two types in any given mature vesicle vary somewhat but usually neither is clearly predominant.

Since both forms are released on the rupture of the vesicle it seems unlikely that they merely represent different developmental stages of the virus, unless it be postulated that development is completed in the intercellular substance. To elucidate this point, and to obtain further information concerning the behaviour of the particles, the infective potential of the two forms is under consideration.

A duality of elementary bodies in electron microscope studies of thin sections of frozen chorioallantoic ectoderm has been observed by Litwin[2], using the virus of feline pneumonitis, and Banfield[3], has reported a similar phenomenon in the unrelated Molluscum contagiosum virus.

F. L. Constable

University New Buildings,
Bacteriology Department,
Teviot Place,
Edinburgh, 8.
June 17.

[1] Porter, K. R., and Blum, J., *Anat. Rec.*, **117**, 685 (1953).
[2] Litwin, J., *Bact. Proc.*, 64 (1958).
[3] Banfield, W. G. Paper presented at a conference on Cytopathology of Virus Infection (N.Y. Acad. Sci. In the press).

## Opsonins as Determinants of Survival in Intraperitoneal Infections of Mice

It has recently been established that the rapidity with which various species of Gram-negative organisms are eliminated from the mouse peritoneum, is entirely dependent on the availability of opsonic factors[1,2]. In the normal mouse peritoneum there are large numbers of mononuclear cells[3] which, once they have engulfed the injected bacteria, destroy them equally rapidly irrespective of their virulence for the mouse. The limiting factor which determines whether overall bacterial multiplication occurs is the rate at which the organisms are phagocytosed, and this is controlled by the supply of opsonic factors. Virulent strains for example of *Salmonella typhimurium* require greater concentrations of serum opsonic factors to promote phagocytosis than do avirulent members of the same species[4]. That these considerations apply to intraperitoneal infections was apparent by the finding that pretreatment of virulent *S. typhimurium* organisms with dilute specific antiserum before injection, greatly altered the capacity of these organisms to cause disease and death in mice[1]. Treatment by dilute antiserum did not by itself affect the viability of the organisms. It seems clear that the trace amounts of antiserum adsorbed by the injected bacteria rendered them susceptible to phagocytosis and this changed the whole balance of the host/parasite interaction.

Specific antibodies can obviously act as opsonic factors, and we had assumed that any opsonic effects in the sera of normal animals could be accounted for by their content of 'natural antibody', specific or otherwise. By extending our observations to the sera of other normal animals we have now found that some sera, particularly those from rats and pigs are extremely effective in preventing the lethal effects of *S. typhimurium* and that this bears no relation to their content of specific O-antibody.

In these experiments the content of antibody directed against the O-somatic antigens of the two bacterial strains, *S. typhimurium* $C_5$ and *Escherichia coli* 145, was determined by hæmagglutination procedures[5]. For this purpose the specific lipopolysaccharides of these two organisms were extracted and purified by the method of Westphal, Lüderitz and Bister[6], sheep red cells were coated with the alkali-treated lipopolysaccharides and the hæmag-

glutination tests performed in accordance with the description of Crumpton, Davies *et al.*[7]. Table 1

Table 1. Effect of Pretreating *S. typhimurium* and *E. coli* with Various Sera, on their Lethality for Mice

| Serum | Challenged with 20 *S. typhimurium* $C_5$ | | Challenged with $10^7$ *E. coli* 145 | |
|---|---|---|---|---|
| | Hæm-agglutination titre of serum | Survivors of 15 mice (28 days) | Hæm-agglutination titre of serum | Survivors of 10 mice (2 days) |
| Horse | 160 | 13 | 240 | 8 |
| Human | 80 | 5 | 60 | 5 |
| Chick | 80 | 4 | — | — |
| Pig | 8 | 14 | 32 | 2 |
| Rabbit | 4 | 0 | — | — |
| Rat | 2 | 11 | 2 | 3 |
| Mouse | 0 | 0 | 2 | 3 |
| Guinea-pig | 0 | 0 | — | — |
| Controls without serum | — | 0 | — | 1 |
| *S. paratyphi B* rabbit anti-serum | 80 | 7 | | |

Table 2. Effect of Inoculum Size on the Protective Action of Pig Serum for *S. typhimurium*

| Inoculum | Orgs pretreated with pig serum | | | Controls | |
|---|---|---|---|---|---|
| | Number of mice in group | Mean death Time (Days) | Mortality at 28 days (per cent) | Mean death Time (Days) | Mortality at 28 days (per cent) |
| 900 | 15 | 4·5 | 100 | 4·5 | 100 |
| 152 | 15 | 5 | 100 | 5 | 100 |
| 48 | 45 | 15 | 66 | 7 | 86 |
| 5 | 60 | 24 | 23 | 10 | 80 |

shows the greatest dilution of each serum which gave visible agglutination of the sensitized cells. All assays were completed within one day's work using a single batch of sensitized sheep cells. The same serum from each animal was then tested for its effect on the lethality of *S. typhimurium* $C_5$ or *E. coli* 145 in mice as follows. The overnight culture of *S. typhimurium* was diluted to give approximately $10^7$ organisms/ml. with 30 per cent serum in saline. Each mixture was incubated at 37° for 10 min. then diluted in saline to give the required numbers of organisms in 0·2 ml. This volume was plated for viable count and also injected intraperitoneally into each of 15 mice. The control series was similarly treated without serum. In Table 1 the numbers, in each group of 15 mice, which survived for 28 days are recorded. It can be seen that considerable protection was afforded to the mice by pretreatment with horse, pig or rat serum in the case of *S. typhimurium* or horse serum with *E. coli*. There is no correlation however, between the specific hæmagglutinating titre of any particular serum and its protective ability for *S. typhimurium*.

A further series of experiments using the same pig serum showed that its remarkable effect is very dependent on the numbers of organisms inoculated. No protection was apparent against *S. typhimurium* when 150 organisms or more were injected into each mouse. With a challenge dose of 5 organisms the 28-day mortality was reduced from 80 per cent in the controls to 23 per cent in the serum-treated group.

It has been shown by Hobson[8] that survivors of *S. typhimurium* infections of mice are very commonly carrying a few of the specific organisms in the spleen which can often be detected for several months. A further consequence of this carrier state is that the mice are highly resistant to a superimposed homologous infection. We have attempted to determine whether mice surviving infection by serum-treated *S. typhimurium* are carriers or not. From a total of 50 survivors, eight were killed and spleens recovered and cultured. The blood from these same mice was pooled, the serum separated and titrated in the hæmagglutination assay as above. The remaining mice were challenged intraperitoneally with approximately 1,000 orgs *S. typhimurium* $C_5$ along with a similar group of normal mice as controls. No specific organisms were recovered from any of the spleen cultures, the hæmagglutination titres were all less than 2 and the remaining mice were just as susceptible to infection as the group of normal mice. It seems likely from these findings that the injected organisms were rapidly killed in the peritoneum before any appreciable number could leak out and reach the spleen. Where larger numbers were inoculated some were able to reach the circulation and settle in the spleen which Schneider and Zinder[9] have shown to be a crucial determinant of subsequent bacterial multiplication and death of the host.

The nature of these serum opsonins and the part, if any, which they play in determining the susceptibility of various species of animal to different infections is now being investigated.

C. R. Jenkin
D. Rowley

The Wright-Fleming Institute of Microbiology,
St. Mary's Hospital Medical School,
Paddington, London, W.2.
July 20.

[1] Whitby, J. L., and Rowley, D., *Brit. J. Exp. Path.* (in the press).
[2] Rowley, D., and Whitby, J. L., *Brit. J. Exp. Path.* (in the press).
[3] Rowley, D., *Nature*, **181**, 1738 (1958).
[4] Rowley, D. (submitted to *J. Exp. Med.*).
[5] Neter, E., *Bact. Rev.*, **20**, 166 (1956).
[6] Westphal, O., Lüderitz, O., and Bister, F., *Z. Naturf.*, **7**, 148 (1952).
[7] Crumpton, M. J., Davies, D. A. L. and Hutchison, A. M., *J. Gen. Microbiol.*, **18**, 129 (1958).
[8] Hobson, D., *J. Hyg.*, **55**, 334 (1957).
[9] Schneider, A. H., and Zinder, N. D., *J. Exp. Med.*, **103**, 207 (1956).

## PLANT PATHOLOGY

### Fungitoxicity of Metal Ions

Many attempts have been made to relate the physical and chemical properties of the metals to the toxicity of their salts. The most successful hypothesis, first proposed by Mathews[1], has been that the toxicity of the ions is related to the electrode potential of the metal. Jones[2] corroborated this hypothesis when he established that the logarithm of the toxic concentration of some 18 metal ions, to the stickleback, *Gasterosteus aculeatus*, and the planarian, *Polycelis nigra*, was approximately a linear function of the standard electrode potential.

However, Danielli and Davies[3] point out that the slope of the above plot gives very poor agreement with the Nernst formula in which observed electrode potential should increase by 0·06 V. for a tenfold rise in concentration of a univalent ion. In fact, assuming that the potentials of the different ions are the same at equally toxic concentrations, the observed increase is 1·0 V. Danielli and Davies consider it unlikely that oxidation by the metal ions is primarily responsible for toxicity and, instead, propose the tightness of covalent bonding of the ion to the surface ionic or thiol groups of the cell to be the important factor.

A measure of the extent of chemical interaction between the metal ion and cell surface is given by the electronegativity of the metal, and Danielli and Davies have shown that Jones's data fit an exponential relationship between the logarithm of the toxic concentration of the metal ion and its electronega-

tivity value. Recently, Danielli[4] has amended this relationship to the linear form:

$$\log (M^{q+})_b = \frac{\varphi F}{RT} + \text{constant} \qquad (1)$$

where $(M^{q+})_b$ is the concentration of metal cation of valency $q$ in the bulk phase which gives the standard toxicity, $\varphi$ is the electronegativity of the metal, $F$ one faraday, $R$ the gas constant, and $T$ the absolute temperature. The arguments in favour of an equation of this type, which assumes that toxicity is dependent on the formation of an unionized complex between metal ions and cell surface components, have been given by Danielli[5] in an earlier paper.

It was decided to investigate further the validity of equation (1) with reference to the fungitoxicity of metal salts many of which, particularly those of the heavy metals, are widely used as fungicides. The toxicities of aqueous solutions of nitrates of potassium, sodium, thallium, silver, barium, strontrium, magnesium, zinc, manganese, beryllium, copper, nickel, mercury, lead, palladium, cerium and yttrium, of the sulphates of lithium and chromium, and of ruthenium chloride and osmium tetroxide were determined against conidia of *Alternaria tenuis*. The solutions were unbuffered and no spore stimulant was required. The standard test-tube dilution spore germination technique[6] was used and the median effective dose ($ED_{50}$) determined visually from the probit regression lines. In Fig. 1 the logarithms of the $ED_{50}$ values for the metal ions have been plotted against the electronegativities of the metals and fair agreement with the linear relationship proposed by Danielli is achieved, although all the metals do not share a common anion. The electronegativity values in Fig. 1 are from Pauling[7], Gordy[8], and Danielli and Davies[3] (chromium, nickel, manganese, mercury, copper and lead) whilst the values for palladium, cerium, ruthenium and osmium are calculated from the first ionization potentials of the metals by the method suggested by Pauling[7]. Because of some uncertainties in Gordy's values for mercury, copper and lead it was decided to use the values of Danielli and Davies[3] for these elements.

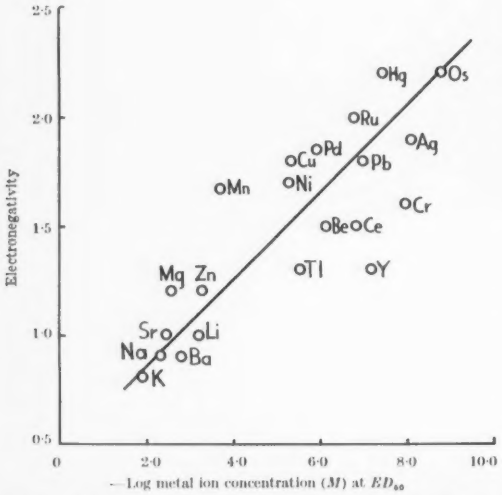



Fig. 1. A plot of the toxicity of metal cations to *A. tenuis* against the electronegativity of the metal

Horsfall[9] has suggested that the fungicidal action of metal ions is primarily due to interaction at the fungal cell surface. The present work lends support to this hypothesis by showing that for the wide range of metallic salts examined, a general relationship of the type of equation (1) holds. Thus, it would appear that the primary toxic action of metal cations is the formation of an unionized complex with surface ionogenic groups, for example, phosphate, carboxyl and SH, and that the different toxicities of the metals can be correlated with the varying strength of surface binding.

A fuller account of this work will be published elsewhere.

E. Somers

Long Ashton Research Station,
University of Bristol.

June 8.

[1] Mathews, A. P., *Amer. J. Physiol.*, **10**, 290 (1904).
[2] Jones, J. R. E., *J. Exp. Biol.*, **16**, 425 (1939); **17**, 403 (1940).
[3] Danielli, J. F., and Davies, J. T. "Advances in Enzymology", **11**, 35 (Interscience, New York, 1949).
[4] Danielli, J. F. in "Surface Phenomena in Chemistry and Biology", 246 (Pergamon Press, London 1958).
[5] Danielli, J. F., *J. Exp. Biol.*, **20**, 167 (1944).
[6] American Phytopathological Society, Committee on Standardization of Fungicidal Tests, *Phytopathol.*, **37**, 354 (1947).
[7] Pauling, L., "The Nature of the Chemical Bond" 2nd Ed. (Cornell Univ. Press, Ithaca, 1948).
[8] Gordy, W., *J. Chem. Phys.*, **14**, 305 (1946).
[9] Horsfall, J. G., "Principles of Fungicidal Action" (Chronica Botanica Co., Waltham 1956).

## HISTOLOGY

### Histochemical Localization of Oxidase Activity in the Mitochondria of the Human Heart

A review of the literature has failed to reveal any definitive histochemical localization of oxidase activity in cardiac as well as skeletal muscle. Recently, several new oxidase techniques have been reported which are capable of precise localizations at the cytochemical level[1,2].

In the present study, frozen sections (8 μ thick) of human heart muscle (obtained at autopsy and surgery) were prepared according to the Adamstone-Taylor cold-knife technique and mounted on chemically clean glass microscopic slides. They were incubated for 30 min. at room temperature (about 24° C.) in a substrate solution containing 10 mgm. N-phenyl-*p*-phenylenediamine (*p*-aminodiphenylamine) (British Drug Houses, Poole, England) and 10 mgm. *p*-methoxy-N-phenyl-*p*-phenylenediamine (variamine blue *B* base) (Carbic Color and Chemical Co., New York). The substrates were first placed in a 50·0 ml. Erlenmeyer flask and dissolved in 0·5 ml. reagent ethanol. Thirty-five ml. distilled water were added followed by 15·0 ml. 0·2 *M tris* buffer *p*H 7·4. The solution was shaken and filtered through folded filter paper into a Coplin jar. Incubating solutions containing 0·001 *M* potassium cyanide as well as 0·001 *M* sodium sulphide were also used. In addition the effect of adding cytochrome *c* (Sigma Chemical Co. St. Louis, Mo.) (20·0 mgm. per Coplin jar) to the substrate solution was observed. Some sections were pretreated with physiological saline for 30 min. prior to incubation. These sections were afterwards incubated in substrate solutions with and without cytochrome *c*.

Following incubation, slides were transferred to a 10 per cent solution of cobaltous acetate in 10 per cent formalin containing 5·0 ml. 0·2 *M p*H 5·2 acetate buffer for 1 hr. They were then washed in running water for about 5 min. and mounted in glycerol-gelatin or polyvinyl pyrrolidone[3].

Fig. 1. Oxidase activity of mitochondria of human heart. Arrow points to intercalated disc ( × 880).

With routine incubations the distribution of dye corresponded to that observed with classical mitochondrial stains (for example, Regaud) (Fig. 1). An interruption of staining was observed in the intercalated disks. The localizations corresponded exactly to those described for heart sarcosomes (mitochondria) by Cleland and Slater[4] who employed the Altmann mitochondrial technique. The distribution of staining also appeared to correspond to the sarcosome pattern as seen with the electron microscope[5]. In some areas intense perinuclear staining, which corresponded to the localization of perinuclear sarcosomes, was observed.

Cyanide and sulphide completely inhibited the reaction, as did pre-incubation in physiological saline. However, the reaction in the saline-treated sections was completely restored by addition of cytochrome *c* to the substrate solution. Addition of cytochrome *c* to routine incubation solutions augmented the staining intensity. Cytochrome oxidase is believed to be associated with mitochondria. Thus, the localization of the staining reaction to mitochondria, as well as the results of the inhibitor and cytochrome *c* tests, would indicate the presence of cytochrome oxidase.

M. S. Burstone

National Institute of Dental Research,
Bethesda 14, Maryland.

[1] Burstone, M. S., *J. Hitochem. and Cytochem.*, **7**, 112 (1959).
[2] Burstone, M. S., *ibid.* (in the press).
[3] Burstone, M. S., *Amer. J. Clin. Path.*, **28**, 429 (1957).
[4] Cleland, K. W., and Slater, E. C., *Quart. J. Micro. Sci.*, **94**, 329 (1953).
[5] Kisch, B., and Bardet, J. M., "The Electron Microscopic Histology of the Heart" (Brooklyn Medical Press, New York, 1951).

## ANIMAL PATHOLOGY

### Sex Chromatin in Cultured Human Tissues

The sex chromatin clump of Barr has been reported as occurring in human female tissues cultured *in vitro* for short periods, and as not occurring in long-established cultures[1,2]. The purpose of this communication is to provide a quantitative summary of the available knowledge in this respect, and to indicate that sex chromatin apparently occurs routinely in suitably stained primary explants, but not in long cultured cells.

Thus far, sex chromatin has been reported in 7 benign outgrowths, in 3 persumably cancerous growths from primary explants of human tissues, and in an unspecified number of primary explants of thyroid tissue[3]. In addition, there have been reports of the absence of sex chromatin in 3 long-term cultures known to be of female origin, that is, H.Ep. No. 1, H.Ad. No. 2 and HeLa[1]. 10 cultures of male origin have been reported as essentially negative[2].

Herein are reported 38 new cases, 29 of female origin, and 9 of male. With the exception of 1 amnion culture, none of the male cells showed characteristic sex clumps. The one exception showed peripheral clumps with an incidence ranging to 12 per cent. Such positive cells were presumed to be contaminants of maternal origin. This case with other amnion cultures will be discussed in greater detail in a subsequent publication.

Of the 29 tissue cultures of female origin 6 were classified as cancer and 23 as benign. The malignant cultures were so called only if they were derived from cancer and showed the appropriate cytological characteristics in *in vitro* growth. This precaution in classification is necessary since many primary explants of cancer tissue give rise only to cytologically benign fibroblast-like cells presumably of stromal origin.

Of the benign cultures 11 were derived from lesions of the uterine cervix, 9 from amnions of female infants, 2 from thyroid lesions, and 1 from an endometrial carcinoma. Of the cultures exhibiting cancer characteristics *in vitro*, 4 stemmed from epidermoid carcinomas of the cervix, and 2 from ovarian carcinomas.

26 of the 29 cultures displayed characteristic peripheral sex chromatin clumps in at least some of the cells. In a number of cases the incidence appeared low, but this may probably be attributed in part to fading of the stain in the older preparations. The negative cases comprised the 2 arising from ovarian carcinomas and 1 arising from an amnion. These cultures were respectively approximately 2½ months, 3½ years, and 2½ years of age. The youngest was in the 9th transfer. The amnion was that designated *Al*85 by Ziteer and Dunnebacke[4]. Samples of the 4 subline strains Nos. *A*1, *A*2, *A*3, and *A*4 were all negative. The sex chromatin positive cultures varied from 2 to 55 days in age. One was carried through 5 transfers, none of the rest through more than 3.

These cases with those previously reported justify the tentative inference that sex chromatin clumps appear invariably in the primary explants of human female tissues—benign or cancerous, but that the sex chromatin feature is eventu .ly lost in later transfers. Further evidence on this point is provided by recent primary explants of H.Ad. No. 1. H.Ad. No. 1 is a tumour of female origin which has been maintained in heterotransplant for almost 3 years and 70 generations[5,6]. The recent explants in tissue culture do exhibit sex chromatin in contrast to the culture previously reported as negative[2]. In this one case a change (loss of sex chromatin) which occurs in tissue culture does not occur in heterotransplant. As previously reported[2], the culture of H.Ad. No. 1 which did not show sex chromatin had been *in vitro* for 1½ months. If our assumption is correct, this would represent the earliest reported loss of sex chromatin. The longest reported survival of sex chromatin appears to be in the case described by Orsi and Ritter[1] as 10 weeks, 9 transfers. Sex chromatin might eventually prove to be a convenient indication that cells cultured *in vitro* have not undergone 'transformation'. On the other hand, while preliminary

cloning experiments do not favour such a possibility, it has not definitely been ruled out that 'transformation' of female cells into established strains is actually a process of selecting the sex chromatin negative cells in the original explant.

Whether or not the 2½-year-old negative female amnion culture reported here had become malignant, it did show a very high mitotic rate (74 mitoses per thousand cells in one count), and moderate variation in nuclear size. It may be true that the hypothetical development of cancer in tissue cultures of benign origin is invariably accompanied by a loss of sex chromatin. Since primary explants of female cancer developing *in vivo* have invariably shown sex chromatin *in vitro* when appropriately stained (but not always in tissue section[2]), it seems that at least in this one respect hypothetical malignant change *in vitro* is not identical with cancer development *in vivo*.

I wish to express my thanks to Dr. J. G. Moore and Mr. W. W. Brandkamp of this University who kindly permitted me to examine a series of their primary explants of cervical tissue; to Dr. T. H. Dunnebacke of the University of California at Berkeley who provided me with stained coverslip samples of a number of cultures; and to Dr. H. W. Toolan of Sloan-Kettering Institute, New York, and Miss M. Tai who provided recent material from H.Ad. No. 1.

These studies were supported by Contract *AT*(04-1)-*GEN*-12 between the Atomic Energy Commission and the University of California at Los Angeles.

Charles P. Miles

School of Medicine,
University of California at Los Angeles.

[1] Orsi, E. V., and Ritter, H. B., *Exp. Cell Res.*, **15**, 244 (1958).
[2] Miles, C. P., *Cancer*, **12**, 293 (1959).
[3] Serr, D. M., Ferguson-Smith, M. A., Ivanov, B., and Paul, J., *Nature*, **182**, 424 (1958).
[4] Zilfer, E. M., and Dunnebacke, T. H., *Cancer Res.*, **17**, 1047 (1957).
[5] Toolan, H. W., *Cancer Res.*, **17**, 415 (1957).
[6] Toolan, H. W. (personal communication).

## Detection of Thyroid Antibodies using Bentonite Particles

Demonstration of circulating thyroglobulin antibodies by the gel diffusion-precipitation technique is a simple procedure which can be used for routine laboratory diagnosis of Hashimoto's thyroiditis[1]. Witebsky and Rose[2], Roitt and Doniach[1] and Owen and Smart[3] have also applied Boyden's tannic acid haemagglutination technique for detection of thyroid antibodies and have demonstrated its greater sensitivity over the precipitation technique. The tannic acid haemagglutination technique is however in our experience, less easy to adapt as a routine procedure because it is complex in its performance and requires minute attention to detail to produce consistent results. A recent report by Bozicevich *et al.* on the successful use of bentonite particles, coated with human γ-globulin, in the detection of the rheumatoid serum factor prompted us to try this substance sensitized with thyroid antigen in the detection of thyroid antibodies.

Thyroid glands removed at autopsy were stripped of their connective tissue, weighed and then blended with twice their weight of physiological saline in a Kenmix blender for 10 min. The resulting homogenate was spun at 4,000 r.p.m. for 10 min. to remove heavy cell debris and fibrous tissue. The supernatant from this centrifugation was then spun in a high-speed centrifuge at 27,000 r.p.m. for 45 min. at 4° C. The supernatant was removed and stored at −20° C. for use in both precipitation and bentonite sensitization tests.

Bentonite is a native colloidal hydrated aluminium silicate, insoluble and free from gritty particles. 2·5 gm. of bentonite (B.D.H.) are added to 300 ml. of distilled water in a flask and thoroughly shaken to mix. 25 ml. of this mixture is transferred to a universal container which is then centrifuged at 3,000 r.p.m. for 10 min.; the supernatant is discarded and replaced with 25 ml. of distilled water, the container shaken vigorously to mix and then recentrifuged. This process is repeated twice. At the end of the final wash the supernatant is removed and 2 ml. of thyroid antigen is added and mixed. After standing for 1 hr. at room temperature the sensitized bentonite particles are washed twice in physiological saline and finally resuspended in 25 ml. of physiological saline.

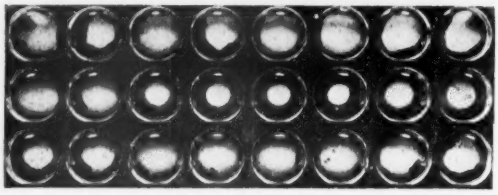


Fig. 1. A positive serum of titre 1 in 250,000, from a case of Hashimoto's disease, compared with two normal sera in rows 1 and 3.

Serial dilutions of the sera to be tested are made, as described by Roitt and Doniach[1], in 'Perspex' agglutination trays. The dilutions range from 1/5 1/2,500,000 and are made with a single pipette using 0·25 ml. volumes and 2 per cent serum saline as a diluent. An antigen inhibition control is included. 0·1 ml. of sensitized bentonite is then added to each dilution and the trays placed in the refrigerator at 4° C. overnight. The pattern of the deposited bentonite particles is read macroscopically and shows the opposite pattern to that encountered with red cell agglutination. Positive agglutination is shown by a small central round button of agglutinated particles and negatives by a thin carpet of bentonite over a wide area of the bottom of the cup (Fig. 1). Weak positives show a central button with a small carpeting around. The end-point is often sharp with no zone of weak positives, and weak positives are only included in the titre if the central button is well defined with little carpeting.

Care must be taken not to jog or disturb the trays after their removal from the refrigerator as this may result in false positives or alteration of titre.

Sensitized bentonite suspensions were used in titrating sera from a variety of cases of thyroid disease and from cases with no thyroid abnormality. In known cases of Hashimoto's disease, with positive precipitin tests, titres up to 1 in 25,000,000 were observed (cf. Owen and Smart[3]). Lower titres of the order 1/5 1/250 were found in a number of other types of thyroid disease. Occasional normal sera gave weak positives at a dilution 1/5. In comparing titres and for controlling the test we use a standard known positive serum and a normal serum together with an antigen inhibition control.

These results suggest that bentonite sensitized with thyroid antigen may be superior to tanned red cells for the detection of thyroid antibodies because of the ease of preparation and the simplicity of the technique.

J. A. M. Ager
M. S. R. Hutt
G. Smith

St. Thomas's Hospital, London, S.E.1.

[1] Roitt, I. M., and Doniach, D., *Lancet*, ii, 1027 (1958).
[2] Witebsky, E., and Rose, N. R., *J. Immunol.*, **76**, 408 (1956).
[3] Owen, S. G., and Smart, G. A., *Lancet*, ii, 1031 (1958).
[4] Bozicevich, J., Bunim, J. J., Freund, J., and Ward, S. B., *Proc. Soc. Exp. biol. and Med.*, **97**, 180 (1958).

## Relation of Invasive Capacity to Passage of Lymphocytic Cells through Cellulose Membrane Filters

In a previous paper[1] it was reported that when three lymphocytic tumours, each with a different capacity to invade host tissues, were grown in Millipore Type *AA* filters (pore size 0·8 ± 0·05μ) (Millipore Filter Corporation, Bedford, Mass.) in double diffusion chambers, the cells of the tumours were unable to penetrate the filter after growing for 60 days in an isologous host. More recent experiments with this filter have shown that while the cells of these tumours are able to pass through the *AA* filter, there is nevertheless a striking difference in the capacity of the cells of an invasive and a non-invasive tumour to penetrate the pores of this membrane and establish a growing cell population on the other side.

The procedures for the construction of diffusion chambers and for introducing the cells into the chambers have been described in detail elsewhere[1]. The cells of the highly invasive lymphocytic leukæmia, *L1210*, and the relatively non-invasive lymphoma, *L1*, were grown as ascites tumours in strain *DBA/2* and strain *A* mice, respectively. Known numbers of tumour cells were placed on one side ('tumour side') of double diffusion chambers, the chambers sealed and inserted by laparotomy into mice of the host strain. Four sets of chambers were prepared as shown in Table 1.

One-half of each set of chambers was removed from the animals after 30 days and the remainder after 120 days. The chambers were opened by cutting away the outer filters with a sharp knife to expose the *AA* filter in the centre and the contents of the two sides of the double chamber. The 'tumour' and 'non-tumour' sides of the chambers were examined and an estimate was made of the amount of tumour tissue on the 'tumour side'. When no growth was visible on the 'non-tumour side', the fluid was centrifuged and the sediment examined microscopically for the presence of cells. The cells on the centre filters were fixed in Orth's fluid and stained with haematoxylin.

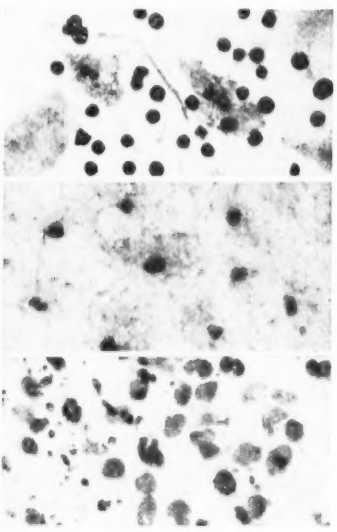


Fig. 1. Top: Spherical, densely stained *L1210* cells have penetrated the Millipore *AA* filter. Normal macrophages are spread out on the filter beneath the tumour cells. Middle and bottom: photographs of 'non-tumour' and 'tumour' side of the same filter on which *L1* cells were grown for 120 days. The middle photograph shows that only macrophages were present on the 'non-tumour' side of the filter even though *L1* tumour cells were growing well on the tumour side of the chamber (bottom).

Table 1.

| Tumour | Set | Chambers (No.) | Tumour age (Days) | Number of cells (millions) | |
|---|---|---|---|---|---|
| | | | | Tumour | Normal |
| *L1* Generation 33 | *A* | 20 | 5 | 1·4 | 0·6 |
| | *B* | 20 | 13 | 1·3 | 0·06 |
| *L1210* Generation 262 | *C* | 20 | 2 | 1·4 | 0·2 |
| | *D* | 20 | 5 | 1·4 | 0·05 |

The initial age of the tumour when placed in the chambers (Table 1) had no detectable effect on their behaviour. The cells of *L1210* penetrated the *AA* filter during the first 30 days in the animal and established a visible amount of growth on the 'non-tumour side' of the chambers. By 120 days the growth of the tumour, which appeared as a mass of white, pasty material, was equal in amount on both sides of the chamber. Examination of the 'non-tumour side' of the *AA* filter in stained whole-mount preparations showed *L1210* cells sprinkled among flattened macrophages on the under surface of the filter (Fig. 1, top).

The behaviour of *L1* differed markedly from *L1210* in that growth of tumour was never macroscopically visible on the 'non-tumour side' of the chambers. It was known from previous experiments that the *L1* tumour did not have the 'growth potential' of *L1210* and, indeed, at 30 days *L1* had not grown to the same mass as *L1210*; but by 120 days, the volume of the growing cell mass of each tumour was roughly equal. At this time, the 'tumour side' of the chambers were one-half to completely full of packed cells. Thus, the mass of tumour tissue did not appear to be related to the penetration of the filter by the tumour cells.

When the sediment of the centrifuged fluid of the 'non-tumour side' of the *L1* chambers was examined with the phase microscope, a few small lymphocyte-like cells with a thin rim of cytoplasm were seen at 30 days; but by 120 days these free-floating cells were no longer present. Although growth appeared to be healthy on the 'tumour side', (Fig. 1, bottom) living tumour cells were observed on the 'non-tumour side' of only one *L1* chamber after 120 days in the animal. With this exception, examination of stained filters failed to reveal viable tumour cells on the 'non-tumour side' of the chambers. instead, the only living cells were the macrophages that spread over the filter surface (Fig. 1, middle). In addition to the macrophages, there were occasional clumps of rounded-up cell ghosts with pyenotic and fragmented remains of nuclei. These appeared to be dead tumour cells and were identified thus because these dead cell clumps do not occur in chambers where only normal peritoneal cells are grown.

These results clearly demonstrate that two tumour-cell populations, given an equal chance, may or may not be able to penetrate an artificial barrier and establish growth at a distant site. It seems probable that some of the same mechanisms which operate in the artificial system of the diffusion chamber, can also operate *in vivo*. One of the factors involved may be the number of cells needed for the establishment of a new focus of growth. The number of dead cells present on the non-tumour side of the *L1* chambers

suggests strongly that the tumour cells were capable of penetrating the filter but in small numbers which were with rare exception, insufficient to establish tumour growth. Similar results were obtained when small numbers of tumour cells were injected directly into mice. We found that inoculation of several thousand *L*1 cells was necessary for the establishment of the tumour in a new host, while Law[2] found that the injection of only ten *L*1210 cells was sufficient for transplantation of that tumour. This difference in the capacity of cell populations to survive may operate in aiding or hindering the establishment of new sites of growth by these tumours while they are growing in the host.

Variation in the capacity of tumour cells to survive has been interpreted as supporting the stem-line concept of tumour populations[3]. If these results are to be interpreted in the light of the stem-line concept, it would be necessary to assume that the *L*1210 tumour is made up almost entirely of stem cells while the *L*1 tumour population is remarkably devoid of stem cells.

Emma Shelton
Mary E. Rice

National Cancer Institute,
Bethesda 14, Maryland.
June 8.

[1] Shelton, E., and Rice, M. E., *J. Nat. Cancer Inst.*, **21**, 137 (1958).
[2] Law, L. W. (personal communication).
[3] Hauschka, T. S., *Trans. N. Y. Acad., Sci.*, Ser. II, **16**, 64 (1953).

## GENETICS

### Semi-Albino: a Third Sex-linked Allelomorph of Silver and Gold in the Fowl

In 1955, among 76,542 chicks hatched from the cross, Brown Leghorn sire by Light Sussex dam, there appeared two males which, instead of having the expected white (silver) coloured down of the Light Sussex, were brown. One was sold before its genetic importance was realized. The other cock when adult was light brown in colour, being of a similar hue to that of Brown Leghorn pullets, with a few black feathers on the wings and a black tail. The black and bright brown colours which are found on the bodies of Brown Leghorn cocks were absent. The bird was strong and vigorous, and lived until killed in 1958.

The absence of silver suggested that this allelomorph (*S*) had mutated either to the allelomorph for gold (*s*) or to some third form. The results of subsequent crosses confirmed that *S* was absent and that a new sex-linked mutant $s^{al}$ recessive to *s* had appeared and that this allelomorph gives semi-albino in the hemizygous females and homozygous males (Table 1). The mutation must have occurred in the Light Sussex dam and not in her ancestors since silver was the gene that had mutated and she herself was not semi-albino. Moreover, since two such cocks appeared in the same year but none was detected among a total of 303,334 chicks hatched between 1956 and 1958, it seems likely that a single mutant occurred during an early stage of oogenesis and that the resulting allelomorph was incorporated in at least two eggs. The mutant can be detected if it arises in the *X*-chromosome of a dam but only in half of those of a sire. Consequently we can say that the mutant has been observed in 2 out of the 379,876 *X*-chromosomes tested.

The semi-albino chicks (all descendants of the second mutant cock) were generally small at hatching, and did not grow as rapidly as the chicks of the other breeds and crosses with which they were reared. The cock chicks were slower in hatching and less active than the pullets. The first feathers were white in colour, but with increasing age many birds developed a buff tinge on the body feathers. This was particularly noticeable in the cocks. In dim light the semi-albino birds had considerable difficulty in seeing, and tended to collide with stationery objects. When adult some of these birds became practically blind due to the development of opacities in the eye lenses. In some cases the whole lens was opaque, while in others the lesions appeared as small spots. The incidence of egg peritonitis in the pullets at between twelve and fourteen months of age was high, and was the most common cause of death.

A similar semi-albino controlled by a sex-linked mutant (*al*) has been reported from America by Hutt[1]. This is almost certainly the same as ours, but since it occurred in Barred Rocks and White Leghorns and not in a stock segregating for silver and gold, its allelomorphism with silver was not detected.

We wish to record the help received from Miss D. G. Kidd, who first observed the unusual appearance of the original bird and was responsible for rearing the experimental chicks, and from Dr. F. T. W. Jordan who carried out post-mortem examinations, and reported on the eye abnormalities.

W. F. Werret
A. J. Candy*

Messrs. J. Bibby and Sons,
Hans Hall Farm,
Willaston, Wirral.

J. O. L. King
P. M. Sheppard

Department of Zoology,
University of Liverpool.
April 17.

* Present address: High Brow, Beechwood Avenue, Frome, Somerset.

[1] Hutt, F. B., "Genetics of the Fowl" (McGraw-Hill, New York, 1949).

Table 1

| Year | Sire | Dam | Sex | Silver | Offspring gold | Semi-albino |
|---|---|---|---|---|---|---|
| 1956 | Mutant ($ss^{al}$) | pure Light Sussex ($S-$) | ♂ | 27 ($Ss$ or $Ss^{al}$) | 0 | 0 |
| 1957 | Mutant ($ss^{al}$) | semi-albino (daughters) ($s^{al}-$) | ♀ | 0 | 12 ($s-$) | 8 ($s^{al}-$) |
| | | | ♂ | 0 | 18 ($s^{al}s^{al}$) | 6 ($s^{al}s^{al}$) |
| | | | ♀ | 0 | 13 ($s-$) | 12 ($s^{al}-$) |
| | | | unsexed | | 5 | 2 |
| 1958 | Semi-albino ($s^{al}s^{al}$) | semi-albino ($s^{al}-$) | ♂ | 0 | 0 | 4 ($s^{al}s^{al}$) |
| | | | ♀ | 0 | 0 | 8 ($s^{al}-$) |
| | | | unsexed | 0 | 0 | 2 |
| 1958 | Semi-albino ($s^{al}s^{al}$) | pure Rhode Island Red ($s-$) | ♂ | 0 | 18 ($ss^{al}$) | 0 |
| | | | ♀ | 0 | 0 | 23 ($s^{al}-$) |
| | | | unsexed | 0 | 0 | 3 |
| 1959 | Semi-albino ($s^{al}s^{al}$) × | Semi-albino ($s^{al}-$) | ♂ | 0 | 0 | 2 |
| | | | ♀ | 0 | 0 | 1 |
| 1959 | Silver ($Ss$) × | Semi-albino ($s^{al}-$) | ♂ | 1 | 0 | 0 |
| | | | unsexed | 0 | 1 | 0 |

## Intraspecific Polyploidy and Evolution of Diverse Morphological Forms in *Convolvulus pluricaulis* Chois.

*Convolvulus pluricaulis* Chois. is a prostrate, spreading, perennial, wild herb commonly found on sandy or rocky ground under xerophytic conditions in northern India. The species is marked by great morphological variability especially in size of the flower. Our cytological studies indicated the existence of intraspecific polyploidy in the species. The present investigation was, therefore, undertaken to study the possible relationship between cytological and morphological forms of the species.

The haploid chromosome number of *C. pluricaulis* with small flowers was determined as nine. Singh[3] reported $n = 10$ for the same species. Our observations along with those of Singh[3] thus indicate the presence of two cytological types in *C. pluricaulis*. Meiotic studies in the large-flowered form revealed it to be a tetraploid with $n = 18$. The presence of eighteen bivalents in 50 per cent of pollen mother cells suggests it to be an allotetraploid. The fact that the remaining 50 per cent of cells showed multivalent associations indicates an autotetraploid or a segmental polyploid origin.

As true allopolyploid forms usually do not show multivalent associations, the tetraploid in *C. pluricaulis* may be considered as an autopolyploid or a segmental polyploid. The general morphological gigantism shown by the tetraploid is an additional support for it to be regarded as an auto—or a segmental-polyploid.

The existence of a natural polyploid series has not so far been recorded in any other species of *Convolvulus*.

Duthie[1] and Hooker[2] have described *Convolvulus pluricaulis* var. *macra* as a large, densely hairy plant with large flowers. The morphological description of *macra*[1,2] is identical with the tetraploid studied in the present investigation. The fact that this variety has been seen to possess double the chromosome number of the small-flowered type further supports the claim of the large-flowered form to be given the rank of a variety. Intraspecific polyploidy has thus played an important part in the evolution of diverse morphological forms in *C. pluricaulis*. The variety *macra* is commonly found in Punjab and Delhi plains and up to an altitude of 3,000 ft. in Kashmir. The small-flowered form which represents the diploid condition is very rare at high altitudes. The distribution of the large-flowered variety is quite in conformity with the general concept that polyploids are found to be more tolerant to extreme ecological conditions than are their diploid relatives.

S. L. Tandon
C. P. Mzalik*

Department of Botany,
University of Delhi,
Delhi 8, India.
June 16.

* Lecturer in Botany, Deshbandhu College, Delhi 19.

[1] Duthie, J. F., "Flora of the Upper Gangetic Plain and of the adjacent Siwalik and Sub-Himalayan Tracts" Superintendent, Government Printing, Calcutta, 1911).
[2] Hooker, J. D., "The Flora of British India" (L. Reeve & Co., Ltd., Kent, 1885).
[3] Singh, B., *Curr. Sci.*, **20**, 105 (1951).

## SOCIAL SCIENCES

### Main Stages of Social Evolution in Man

Prof. Grahame Clark has given tentative estimates of the population of England and Wales, or Britain, in early times. These figures, together with the somewhat firmer estimates for the historical period[1] form the basis of Figs. 1 and 3. L. S. Palmer gives tentative estimates of the number of general kinds of materials used for making things[2]. These form the basis of Figs. 2 and 4.

The difficulties of attempting to quantify states of cultural development are well known. But of the graphs below, we may say, in the words of Stuart Piggott in another connexion (ref. 3 and personal communication, 1959), "While obviously open to criticism in detail at almost every point, it is felt that the broad pattern is sound, and that some sort of graphic statement, however tentative, must be attempted . . ." Whereas the slopes of these curves are liable to revision in the light of future knowledge, it is probable that the main 'kinks' are real, which for the present purpose is what matters. It is hoped that a small group of specialists may soon give this topic the attention which it seems to deserve.

The 600,000 or so years of the existence of tool-making man are occupied, at the zoological level, by a number of different species. But at the sociological level there are, perhaps, two or three main stages of social evolution which may be generally recognized.

Figs. 1 and 2 suggest a division into a long and relatively static stage, followed by a short stage of rapid change. By replotting on a larger scale, however, the beginning of the recent upsurge can be traced back to the introduction to Britain of agri-

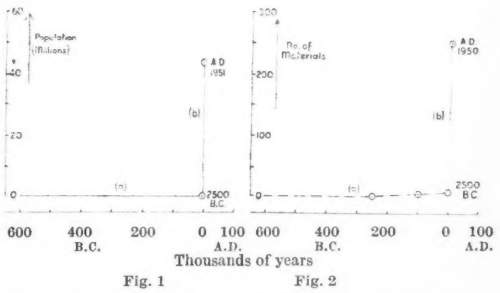



Fig. 1. Plot of population of England and Wales, against time; stage (*a*) after Clark (ref. 1). On this scale, the population during stage (*a*) is indistinguishable from the zero level

Fig. 2. Plot of estimated number of general kinds of materials used for making things, against time; after Palmer (ref. 2). During earliest times and ice ages, the level for populated areas replaces that for Britain

culture and the neolithic way of life. This probably occurred about 2,500 B.C.[3]. Just before that, the total population of Great Britain may have been of the order of 4,500, with rather more than 3,000 people in England and Wales[1]. The 'number of materials', *sensu* Palmer, may have been about 11.

This primary division is, in fact, into (*a*) the food-gathering stage, and (*b*) the food-producing stage[4].

The best tripartite classification seems to be obtained by splitting up stage (*b*), in which such great change occurred, rather than by subdividing the longer stage (*a*).

At least one further and distinct step in social evolution has undoubtedly taken place since 2,500 B.C. In recent years, many writers have compared the inception of agriculture with the events of the past few centuries; with the 'industrial revolution', or that plus the scientific and technological developments since. These include G. P. Thomson and J. H. Plumb[5], besides J. Hawkes, C. Hawkes and V. G. Childe. They rank the beginning of agriculture as possibly even as important as our recent changes. Figs. 1 and 2 depict the beginning of agriculture as the more important. There is at least the clear implication that no such rapid and fundamental social changes have occurred in between.

Figs. 3 and 4, in which the ordinates are logarithmic[6], are of interest in this connexion. On the right-hand side of each plot, a new step is seen to arise, corresponding to the changes of recent centuries. But

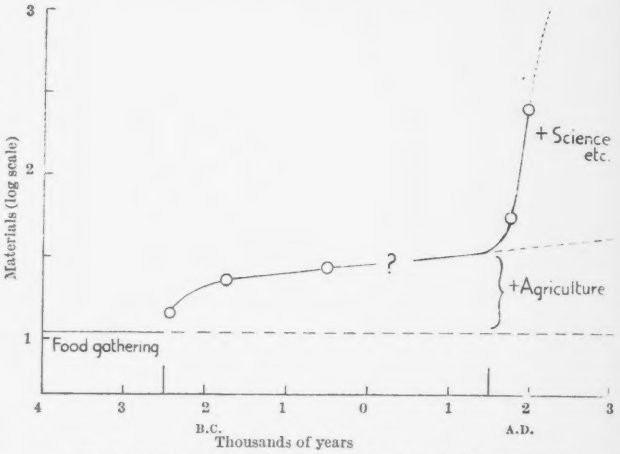



Fig. 4. Logarithm of the estimated number of materials, against time (schematic); adapted from Palmer (ref. 2)

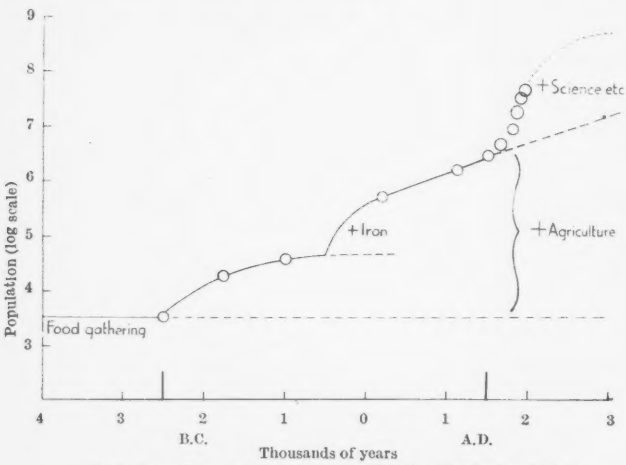



Fig. 3. Logarithm of the estimated population of England and Wales, plotted against time (schematic); earlier data adapted from Clark (ref. 1). (With iron tools, agriculture became possible over a much larger area)

an important general inference of these plots, and of collateral historical data, is that the recent and rapid social changes—of which contemporaries themselves have been conscious since the 1760's[5]—must be regarded as having commenced at least by about A.D. 1500.

A further general inference is this: that we seem to be not yet past the middle of a long sigmoid curve of social evolution. (In Britain the population curve is turning over; but not yet the curve for scientific knowledge[7].) Barring accidents, we may expect as much further social development in the next 500 years as in the past 500. After some such period, consolidation, rather than advance, may again set in.

Since A.D. 1500, there have been conspicuous changes in trade, transport and finance; in industrial techniques, experimentation and scientific theory; in political structure, communication and education—among other aspects. Recently, we have more power-driven machinery and modern science. It is not easy to pick the right word to describe the long-term social transformation in the midst of which we are at present living. But perhaps we may say that we are now entering the 'scientific stage of human society.

Thus, in conclusion, and aided by tentative quantitative data, it is proposed to recognize as the three main stages, to date, of the social evolution of man: (1) *The food-gathering stage*, commencing more than half a million years ago. (2) *The proto-agricultural stage*, commencing locally, near the junction of Asia and Africa, some ten thousand years ago, and reaching Britain about four and a half thousand years ago. (3) *The scientific stage*, commencing in Britain and elsewhere about A.D. 1500; but this third main stage is a stage of social evolution of which even the more advanced countries are still only upon the threshold.

Max Pettersson

Brunel College of Technology,
London, W.3.

June 3.

[1] Clrak, J. G. D., "Archæology and Society" (London, 1957). Russell, J. C., "British Medieval Population" (Albuquerque, 1948).
[2] Palmer, L. S., "Man's Journey through Time" (Lond on, 1957).
[3] Piggott, S., "The Neolithic Cultures of the British Isles" (Cambridge, 1954).
[4] Childe, V. G., "What Happened in History" (Harmondsworth, 1942).
[5] Plumb, J. H., "England in the Eighteenth Century" (Harmondsworth, 1950). Thomson, G. P., "The Foreseeable Future" (Cambridge, 1955).
[6] Pettersson, M. L. R., "A Survey of Science" (London, 1958).
[7] Price, D. J., *Discovery*, 17, 240 (1956).